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Original Article

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Estimation of Serum Calcium, Uric acid and Lipid Profile Levels in Women with Normal Pregnancy and Pre-eclampsia in Rohilkhand Region of Uttar Pradesh

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Abstract

Background and Objective: Incidence of preeclampsia in India is reported to be 8-10% of pregnancies. It is a non-convulsive form of hypertensive disorder of pregnancy. The present study has been undertaken to estimate and compare the serum values of calcium, uric acid, triglycerides, total cholesterol HDL-C and LDL-C between l non pregnant healthy women, normotensive pregnant women and pre-eclamptic women. *Materials and Methods:* Study includes 120 women divided into three groups. Estimation of serum calcium, uric acid, triglycerides, total-cholesterol and HDL-C were analyzed by O-Cresolphthalein-Complexone , Modified Trinder Peroxidase, CHOD-PAP and Phosphotungstic acid precipitation method, using Erba Chem-5 plus semi-autoanalyser. *Results:* Mean serum calcium and HDL-C levels were significantly decreased in group-C and group-B in comparison to group-I, $p<0.05$. The mean serum concentration of uric acid in pre-eclamptic pregnant women (group-C) was significantly elevated than normotensive pregnant women (group-B) cases and healthy non-pregnant women (group-A). The mean triglyceride, and LDL-C levels were significantly increased in group-C as compared to group-B, $p<0.05$. But there was no difference in the mean values of total-cholesterol between cases (group-B and group-C) and control (group-A), $p>0.05$. *Conclusion:* Women having pre-eclampsia had low levels of serum calcium, elevated the serum uric acid and disturbed lipid profile. These levels may have cause and effect relationship with these disorders.

Keywords: Pregnancy; Pre-Eclampsia; Calcium; Uric Acid; Lipid Profile; Women.

Introduction

Pre-eclampsia is a non-convulsive form of hypertensive disorder of pregnancy [1]. Incidence of preeclampsia in India is reported to be 8-10% of pregnancies. The diagnosis of pre-eclampsia (International Society for the Study of Hypertension in Pregnancy) is determined by the presence of elevated blood pressure combined with significant proteinuria (≥0.3 g / 24 hours) after the 20th week of gestation in a previously normotensive, non-proteinuric patient [2]. These disorders are associated with adverse prenatal outcomes such as stillbirth, preterm and small for gestational age babies [3,4]. In India majority of the cases of pre-eclampsia are the patients belonged to poor socio-economic class and have not received proper medical attention during their antenatal period [5].

Calcium is one of the most abundant elements in the human body. Deficiency of calcium may lead to tetanic convulsions, bleeding diathesis, capillary haemorrhages, tissue exudation and osteomalacia [6]. Some studies have concluded that the increase in the intracellular calcium causes vasoconstriction, increase in the peripheral resistance and therefore, an increase in the blood pressure [7]. Epidemiological and clinical studies have shown that an inverse

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relationship exists between calcium intake and development of hypertension in pregnancy [8]. Many trials have been conducted to observe the protective effect of preventive calcium supplementation in pregnant women . A previous review by Hofmyer et al. has shown that calcium supplementation during pregnancy had a significant effect in reducing risk of gestational hypertension and pre-eclampsia [9,10].

Uric acid (2, 6, 8-trihydroxypurine) is the end product of purine metabolism and its elevated level induces endothelial dysfunction and may induce hypertension and vascular disease [11]. An association between elevated serum uric acid levels and preeclampsia was first reported by Slemons and Bogert in 1917 [12]. In women who go on to develop preeclampsia, uric acid concentration is elevated as early as 10 weeks of gestation, at a time much earlier than clinical presentation of the disorder. There are several proposed mechanisms for elevation of uric acid in the pre- eclampsia, such as abnormal renal clearance, increased tissue breakdown, acidosis and a rise in the activity of the xanthine oxidase/ dehydrogenase enzyme [13].

The association of alteration in serum lipid profile in essential hypertension is well documented. Various studies claim that abnormal lipid synthesis leading to increase of thromboxane level and the decrease of prostaglandin levels as well as the imbalance of lipid peroxidase and antioxidants is responsible for pre-eclampsia. There is a positive correlation between serum triglycerides and systolic blood pressure as well as diastolic blood pressure in pre eclampsia cases. Hormonal imbalance leading to altered lipid profile in serum is assumed to be the prime factor in etiopathogenesis of pregnancy - induced hypertension (PIH) [14,15].

In spite of numerous studies; the etiology of pre-eclampsia has not yet been fully elucidated. According to many authors in recent studies observes that the changes in levels of serum calcium, uric acid and lipid profile appear to be of immense value in understanding the pathogenesis of pre-eclampsia. The present study has been undertaken to evaluate and compare the changes in serum levels of calcium, uric acid and lipid profile in healthy non-pregnant women, normal pregnant women and in pre-eclamptic women in Rohilkhand region of Uttar Pradesh.

Materials and Methods

The study was conducted at the Department of Biochemistry, Rohilkhand Medical College and Hospital (RMCH), Bareilly, Uttar Pradesh. The duration of study was 12 months, from March 2015 to February 2016. Total 120 women were recruited for this case control study. They were divided in the following three groups, each group consisting of 40 subjects. *Group A:* Comprised healthy non-pregnant women with age's ranges from 22-35 years taken as controls, *Group B:* comprised of normotensive pregnant women with same age groups, receiving antepartum care at the out patients department and *Group C:* consisted of pre-eclamptic women with similar age groups who were admitted to the Department of Obstetrics and Gynecology, RMCH, Bareilly. All cases were selected by taking a detailed medical history, physical examination and other relative investigations. While selecting the subjects, care was taken that none of them was suffering from diabetes mellitus, cardio-vascular diseases, renal diseases, chronic hypertension, and co-agulation disorders. Before performing the various tests subjects consent had been taken. All the procedures reported here in the study have followed the guidelines approved by the locally appointed ethical committee.

Pre-eclampsia was defined as development of blood pressure > 140/90 mmHg after 20 weeks of gestation and proteinuria of ≥300 mg as confirmed by 24h urine collection in women with no known history of hypertension, renal disease, and endocrine abnormalities and had single pregnancy and had no family history of lipid or carbohydrate disorders (Lampinen et al 2008).

Blood pressures of our selected subjects were measured by standard mercury sphygmomanometer and venous blood samples were collected from antecubital vein after an overnight fasting from all participant's with aseptic precautions. Blood samples were allowed to clot at room temperature and the serum was separated by centrifugation. The estimation of these parameters was carried out within 4-6 hrs. The following tests were done in each sample during the study.

a. Serum Calcium by O-Cresolphthalein-Complexone method [16].
b. serum uric acid by Modified Trinder Peroxidase method [17].
c. Serum Triglycerides was measured by GPO-PAP method [18].
d. Serum Total Cholesterol by CHOD-PAP method [19].
e. HDL-Cholesterol Estimation by Phosphotungstic acid precipitation method [20].
f. Estimation of Serum LDL cholesterol.

Indirect method has been used in accordance with the outline of Freidewald's Formula. (Freidewald W.T. et al 1972).

The value of LDL cholesterol is calculated as

LDL-Cholesterol= Total Cholesterol – [(Triglycerides/5) + (HDL-Cholesterol)]

Statistical Analysis

Data were presented as mean ± SD. A student's unpaired t-test was used for cross sectional comparisons of continuous variables between the 2 groups. The results were considered statistically significant when the probability of the null hypothesis was less than at least 5% ($p < 0.05$).

Results

Of the 40 pre-eclamptic patients 24 were primigravida (60%) and 16 were multigravida (40%). The mean gestational age of pre-eclamptic women in group-C was statistically significant in comparison to normotensive pregnant women in group-B (31.17 ± 4.08 week vs. 33.00 ± 4.37 week) [Table 1, Figure 1.A, B].

From Table 2 and Figure 2 we compared the results of mean systolic, diastolic and mean arterial blood pressure (MAP) of Group-B women with Group-A women and Group-C women with group-B women (Table 2, Figure 2). The mean ± SD systolic, diastolic and mean arterial blood pressure (MAP) of Group-B women were significantly higher than Group-A women (126.0 ± 6.8 vs 120.0 ± 9.3 mm of Hg, 78.1 ± 7.5 vs. 70.8 ± 8.3 mm of Hg and 96.7 ± 5.48 vs. 85.5 ± 6.68 mm of Hg) and Group-C women were significantly higher than Group- B women (174.2 ± 13.1 vs. 126.0 ± 6.8 mm of Hg, 105.4 ± 7.3 vs. 78.1 ± 7.5 mm of Hg and 128.0 ± 6.5 vs. 92.7 ± 5.48 mm of Hg).

Table 3, Figure 3. A & B shows significantly lower mean serum calcium & HDL-C in Group-B and Group-C ($p < 0.05$) compared to Group-A. The mean serum concentration of uric acid in normotensive pregnant women (Group-B) cases was significantly higher in comparison to healthy non-pregnant women (Group-A). But the mean value of serum uric acid in pre-eclamptic pregnant women (Group-C) was significantly elevated than normotensive pregnant women (Group-B) [6.9 ± 0.54 mg/dl vs 5.17

Table 1: Showing gestational weeks and Parity of the study groups

Subjects		Group-B (40)	Group-C (40)	P value
Estimated Gestational weeks	24-28	07(17.5%)	12 (30 %)	
	29-33	15 (37.5 %)	13 (32.5 %)	
	34- 38	18(45 %)	15(37.5 %)	
	Mean ± SD	33.00 ± 4.37	31.17 ± 4.08	Gr. B vs Gr.C $p < 0.05$
Parity	Primi	28 (70%)	24 (60%)	
	Multi	12 (30%)	16 (40%)	

Table 2: Mean systolic, diastolic and mean arterial pressure (MAP) for the three groups of participants.

Blood Pressure (mm of Hg)	Group-A	Group-B	Group-C	P value
Systolic (mean ± SD)	120.0 ± 9.3	126.0 ± 6.8	174.2 ± 13.1	Gr. A vs Gr.B $p < 0.05$, Gr.B vs Gr.C $p < 0.05$
Diastolic (mean ± SD)	70.8 ± 8.3	78.1 ± 7.5	105.4 ± 7.3	Gr. A vs Gr.B $p < 0.05$, Gr.B vs Gr.C $p < 0.05$
Mean arterial pressure (mean ± SD)	85.5 ± 6.67	96.7 ± 5.48	128.0 ± 6.5	Gr. A vs Gr.B $p < 0.05$, Gr.B vs Gr.C $p < 0.05$

Table 3: Showing serum calcium, uric acid & lipid profile values in study groups

Parameters	Group-A (n=40)	Group-B (n=40)	Group-C (n=40)	Statistical relationship of Gr-B & Gr-C with Gr-A
Calcium (mg/dl)	9.60 ± 0.70	8.80 ± 0.65	8.22 ± 0.63	P < 0.05 in Gr-B; P< 0.05 in Gr C
Serum Uric acid (mg/dl)	3.89 ± 0.64	5.18 ± 0.94	7.1± 0.54	p < 0.05 in Gr.B & p < 0.05 in Gr Gr.C
Triglyceride (mg/dl)	174.02 ± 11.2	179.6 ± 14.1	203.22 ± 15.7	P < 0.05 in Gr- B; P > 0.05 in Gr-C
Total Cholesterol (mg/dl)	190.98 ± 13.3	197.48 ± 14.4	195. 74 ± 13.8	P>0 .05 in Gr-B; P > 0.05 in Gr-C
HDL-C (mg/dl)	53.58 ± 4.43	49.22 ± 6.25	47.94 ± 5.7	P <0.05 in Gr-B; P < 0.05 in Gr-C
LDL-C (mg/dl)	102.6 ± 13.8	112.3 ± 17.23	107.09± 16.31	P < .05 in Gr-B; P > 0.05 in Gr. C

± 0.94 mg / dl, $p < 0.05$. Triglyceride mean value was significantly higher ($p < 0.05$) in Group-B cases as compared to Group-A; but non significantly higher ($p > 0.05$) in Group-C in comparison to Group-A. We also observed non significant ($p > 0.05$) difference between mean values of total cholesterol between cases (Group-B and Group-C) and control (Group-A). The mean value of LDL-cholesterol which was highly significant ($p < 0.05$) in Group-B cases and non significant ($p > 0.05$) in Group-C cases in comparison to Group-A cases.

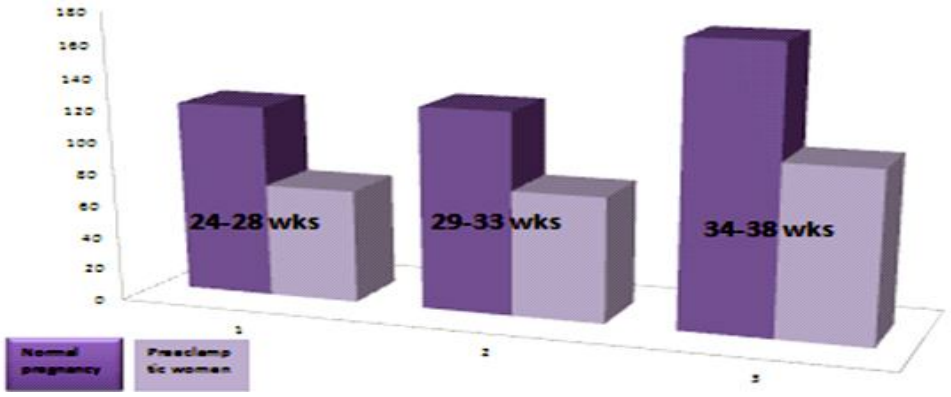

Fig. 1A: Showing number of women in normal pregnancy and pre-eclamptic cases along with gestational weeks

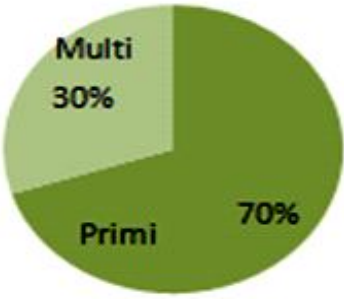

Fig. 1B: Showing number of primi and multi women in normal pregnancy

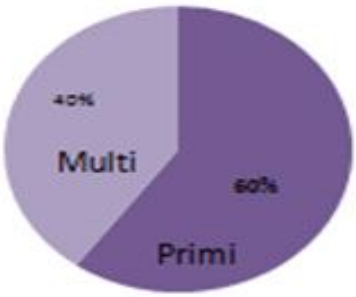

Fig. 1C: Showing number of primi and multi pre-eclamptic women

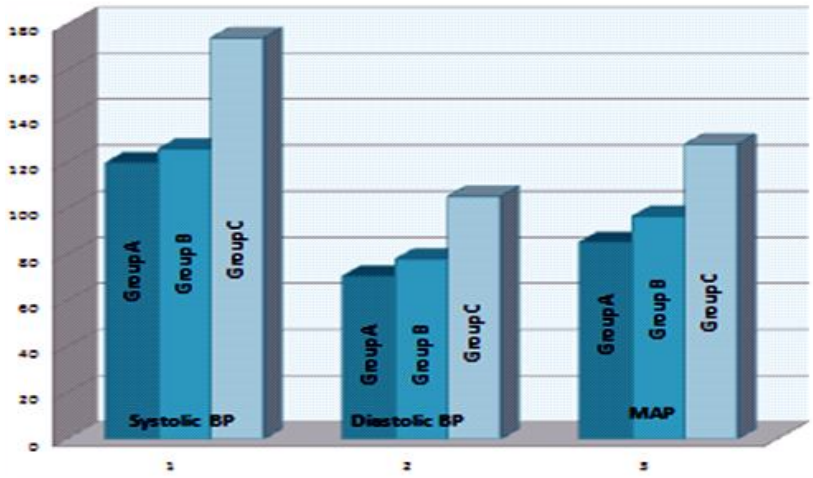

Fig. 2: Showing mean systolic, diastolic and mean arterial pressure (MAP) of three different studied Groups.

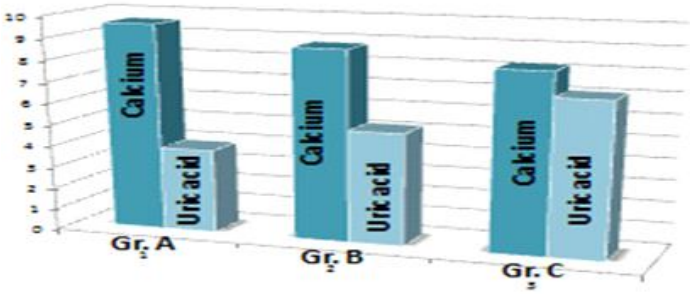

Fig. 3A: Showing serum calcium and uric acid levels in three different Groups.

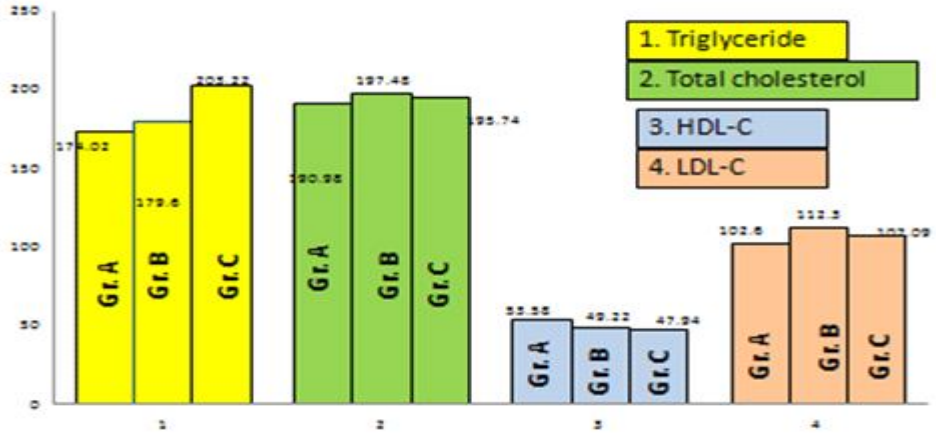

Fig. 3B: Showing serum triglyceride, total cholesterol, HDL-C and LDL-C levels in three different studied Groups.

Discussion

In this study the mean ± SD gestational ages of Group B and group C cases were 33.00 ± 4.37 week and 31.17 ± 4.08 week and also majority of the patients (65%) were primigravida (Table 1). The mean gestational age was highly significant in pre-eclamptic women (Group-C) in comparison to normotensive pregnant women (Group-B) ($p < 0.05$). The mean systolic, diastolic and mean arterial blood pressure (MAP) in pre-eclamptic women (Group-C) was significantly higher than normotensive pregnant women (Group-B) and non-pregnant healthy women (Group-A). These results are in agreement with study of other authors [21,22].

From Table 3 we observed that mean serum calcium value was highly significant between non pregnant healthy women (Gr. A) with pregnant healthy women (Gr. B) and preeclamptic women (Gr. C). This finding matches with previous studies conducted by Idogun E.S. et al. [23], J. Moodley et al. [24], Punthumapol C et al [25]. This result supported the hypothesis that low serum calcium level might be a cause in the development of pre-eclampsia. The effect of serum calcium on changes in blood pressure could be explained by the level of intracellular concentration of calcium[25]. Belizan [26] hypothesized that a low calcium intake results in high parathyroid hormone levels and increased membrane permeability. As a result, calcium is released from the mitochondria and it enters the cytoplasm, thus resulting in increased intracellular free calcium levels and decreased serum calcium levels. The elevation of cytoplasmic calcium levels triggers smooth muscle contraction, thus resulting in vascular constriction and increased blood pressure [27]. In our study majority of the pregnant women in Group-B and Group-C are low socioeconomic status. Socioeconomic status may be correlated with calcium intake. Women from the low income group were more likely to have less than the recommended dietary allowance (RDA) for calcium. However, the present finding was contradictory to some other studies where the mean serum calcium levels in pre-eclamptic women was not different from normal pregnancy [28,29].

The mean serum uric acid levels in the present study significantly lower in Group-A (non-pregnant healthy women) than Group-B (normotensive pregnant women), $p < 0.05$. Our results agree with previous findings of some authors [30,31]. A decreased glomerular filtration rate may contribute to an increased uric acid, but this likely occurs later

in pregnancy closer to the time of pre-eclampsia diagnosis [31]. In the present study, we also found significantly elevated mean serum uric acid level in pre- eclamptic women (Group-C) compared to normotensive pregnant women (Group-B). Similar results were observed by other authors [25,32]. Hyperuricemia is believed to be resulted from decreased renal excretion as a consequence of pre-eclampsia, also results from increased production secondary to tissue ischemia and oxidative stress [32]. Soluble uric acid impairs nitric oxide generation in endothelial cells. Thus hyperuricemia induces endothelial dysfunction and may induce hypertension and vascular disease [32]. But in some authors namely Salako BL et al., Weerasekera DS et al. [33,34] did not find any significant difference in mean serum uric acid levels between pre- eclamptic women and normotensive pregnant women.

The current study we analyzed, the role of lipid parameters in pre-eclampsia cases and found that the patients of Group-C (preeclampsia) have significant difference in serum triglyceride as compare to Group-A (control) subject ($p < 0.05$). In this study the mean ± SD serum triglyceride of Group-C participant was (203.22 ± 15.7) more than the mean ± SD serum triglyceride of Group-A (174.02 ± 11.2) participants. These findings are in agreement with work of many authors [21,35,36]. The principle modulator of this increase in triglyceride is esterogen, as pregnancy is associated with hyperestrogenaemia. Esterogen inhibits the hepatic lipid oxidation so the net effect is increased delivery of free fatty acids into hepatic biosynthesis of endogenous triglycerides which carried by VLDL (Jayanta et al. 2006) [35]. But some authors believed that the increase level of triglycerides in preeclampsia is probably not due to hyperesterogenaemia as the levels of esterogen decreases in pre-eclampsia. Another hypothesis for increase level of triglycerides in pre-eclampsia is that hyper-triglyceridemia is probably a consequence of competition between the substrates chylomicron and very low-density lipoprotein cholesterol for the enzyme lipoprotein lipase. Classically, chylomicron clearance occurs in two sequential steps: (a) Triglyceride hydrolysis by the enzyme lipoprotein lipase, (b) Uptake of the remnant by the liver. Delay in the second step leads to accumulation of remnants in plasma and is generally thought to represent the atherogenic risk of hyper-triglyceridemia. Elevation in triglyceride, found in preeclampsia is likely to be deposited in predisposed vessels, such as uterine spiral arteries and contributes to the endothelial dysfunction, both directly and indirectly through generation of small dense low-density lipoprotein cholesterol (Sattar et al. 1997) [37].

But we could not observe any significant change in maternal serum TC (total cholesterol) level in these studied groups. This finding is similar to previous studies conducted by NAF Islam et al [21], jayanta et al [38], Sattar et al [37]. From Table 3; Figure 3.B we also observed that the increase in HDL-C and decrease in LDL-C levels is due to hyperestrogenaemia. But in preeclampsia, the estrogen level is decreased, so reduced serum HDL-C level and increased serum LDL-C levels were observed. This finding is supported by other authors [21,39].

Conclusion

Analysis of the results of the present works, it is clear that the women who develop pre-eclampsia had low level of maternal total serum calcium, elevation of the serum uric acid level and disturbed lipid profile due to abnormal lipid metabolism. This association may be significant in understanding the pathological processes of pre-eclampsia and may help in developing strategies for prevention and early diagnosis of pre-eclampsia and other .

References

1. Montan S, Sjoberg N, Svenningsen N. Hypertension in pregnancy—fetal and infant outcome: a cohort study. Hyper tens pregnancy. 1987; 6: 337-48.
2. Brown MA, Lindheimer MN, De Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hyper tens pregnancy. 2001; 20: 9-24.
3. Habli M, Levine RJ, Qian C, Sibai B. Neonatal outcomes in pregnancies with preeclampsia or gestational hypertension and in normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation. Am J Obstet Gynecol. 2007; 197(4): 406 e401-407.
4. Ananth CV, Basso O: Impact of pregnancy-induced hypertension on stillbirth and neonatal mortality. Epidemiology. 2010; 21(1): 118-123.
5. Surraya Halimi, Syed Muhammad Ashhad Halimi. Eclampsia and its association with external factors. J Ayub Med Coll Abbottabad. 2010; 22(3).
6. Indumati V et al. Role of serum electrolytes in PIH; Journal of Clinical and Diagnostic Research. 2011 Feb; 5(1): 66-69.
7. Belizan J.M., Villar. J., Repke J. The relationship between calcium intake and pregnancy induced

hypertension: up-to-date evidence. Am. J. Obstet. Gynecol. 1988; 158: 898-902.

8. Ortega RM, Martinez RM, Lopez-Sobaler AM, Andres P, Quintas ME. Influence of calcium intake on gestational hypertension. Ann Nutr Metab. 1999, 43(1): 37-46.
9. Hofmeyr GJ, Duley L, Atallah A. Dietary calcium supplementation for prevention of pre-eclampsia and related problems: a systematic review and commentary. B jog. 2007; 114(8): 933-943.
10. Hofmeyr GJ, Atallah AN, Duley L: Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. Cochrane Database Syst Rev. 2006; 3: CD001059.
11. Sukonpan K, Phupong V. Serum calcium and serum magnesium in normal and pre-eclamptic pregnancies. Arch Gynecol Obstet. 2005; 273: 12-16.
12. Slemons JM, LJ Bogert: The uric acid content of maternal and fetal blood. J Biol Chem. 1917; 32: 63.
13. Bain bridge SA, Roberts JM. Uric acid as a pathogenic factor in pre- eclampsia. Placenta. 2008; 29: S67-S72.
14. Robson, S.C. Hypertension and renal disease in pregnancy; In: Dew Hurst's Textbook of Obstetrics and Gynecology for postgraduates, Ed. Edmonds, D.K., 6th ed. Blackwell Science Ltd., 1999; New York, p 167- 9.
15. Karl W, Birgit W, Michael M. H et al. Triglyceride Rich Lipoproteins Are Associated with Hypertension in Pre-eclampsia. The Journal of Clinical Endocrinology & Metabolism. 2003; 88(3): 1162-1166.
16. Gindler EM & King J.D. Am J. Clin Pathol. 1972; 58: 376.
17. Kabasakalian P., Kalliney S. and Wescott A. Clin. Chem. 1973; 19: 522.
18. McGOWAN, M. W. ARTISS, J. D. STRANBERG, D. R. ZAK, B. A. Peroxidase coupled method for the colorimetric determination of serum Triglycerides. Clin.Chem. 1983; 29: 538-542.
19. Allian, C.C., L.S.Poon, C.S.Chan, W.S.Richmond and P.C. Fu, Enzymatic determination of total serum cholesterol. Clin.Chem. 1974; 20: 470-475.
20. Burstein M., Scholnic H. R., Morfin R. J. Lipid Res. 1970.
21. NAF Islam et al. Study of serum lipid profile in pre-eclampsia and eclampsia. Faridpur Med. Coll. J. 2010; 5(2): 56-59.
22. Das et al. Evaluation of Serum Calcium and Lipid Profile in Womenwith Pre-eclampsia, Eclampsia and Normal Pregnancy in Rohilkhand region of Uttar Pradesh. NJIRM. 2011 Oct-Dec; 2(4): 15-22.
23. Idogun E.S et al. extra cellular calcium and magnesium in preeclampsia and eclampsia. African journal of reproductive health. 2007; 11(2): 89-94.
24. J. Moodley et. al. Serum calcium ion concentrations in eclampsia. South African Medical research Council, Johannesburg. 1987; 72: 382-385.
25. Punthumapol C et al. J Med Assoc Thai. 2008; 91 (7): 968-973.
26. Belizan J.M., Villar. J., Repke J. The relationship between calcium intake and pregnancy induced hypertension: up-to-date evidence. Am. J. Obstet. Gynecol. 1988; 158: 898-902.
27. Standley CA, Whitty JE, Mason BA, Cotton DB. Serum ionized magnesium levels in normal and pre-eclamptic gestation. Obstet Gynecol. 1997; 89: 24-7.
28. Ingec M, Nazik H, Kadanali S. Urinary calcium excretion in severe pre-eclampsia and eclampsia. Clin Chem Lab Med. 2006; 44: 51-3.
29. Hayder A.H.AL-Biati. Serum Lipid Profile And Uric Acid Concentration During Pregnancy. Diyala Journal of Medicine. 2011 December; 1(2): 90-94.
30. Dehghan, A., van Hoek, M., Sijbrands, E.J., Hofman, A., Witteman, J.C.. High serum uric acid as a novel risk factor for type 2 diabetes. Diabetes Care 2008; 31: 361-2.
31. Williams KP, Galerneau F. The role of serum uric acid as a prognostic indicator of the severity of maternal and fetal complications in hypertensive pregnancies. J Obstet Gynaecol Can. 2002; 24: 628-32.
32. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, et al. Hyperuricemia induces endothelial dysfunction. Kidney Int. 2005; 67: 1739-42.
33. Salako BL, Odukogbe AT, Olayemi O, Adedapo KS, Aimakhu CO, Alu FE, et al. Serum albumin, creatinine, uric acid and hypertensive disorders of pregnancy. East Afr Med J. 2003; 80: 424-8.
34. Weerasekera DS, Peiris H. The significance of serum uric acid, creatinine and urinary microprotein levels in predicting pre-eclampsia. J Obstet Gynaecol. 2003; 23: 17-9.
35. Powers RW, Evans RW, Majors AK. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. Am J Obstet Gynecol. 1998; 179:1605-1611.
36. Enquobahrie, DA, Williams MA, Butler CL, Miller RS, Luthy DA. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. Am J hypertens. 2004; 17(7): 574-81.
37. Sattar, N., A. bendomir, C. Berry, J. Shepherd, I. Greer and C. J. Packard. Lipoprotein subfraction concentrations in pre-eclampsia: Pathogenic parallels to atherosclerosis. Obstet. Gynecol. 1997; 89: 403-408.
38. Jayanta, D., K.M. Ananda and K.S. Pradip. Study of serum lipid profile in PIH. Indian J. Clin. Biochem. 2006; 21: 165-168.
39. Gratacos E, casals E, Gomez O, et. al. Increased susceptibility to low density lipoprotein oxidation in women with a history of pre-eclampsia. Br J of Obst & Gynae. 2003; 110(4): 400-4.

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Original Article

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Screening of β-Thallasemia in Tribal Population of Hingoli District

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Abstract

The cross sectional study was performed to find out the incidence of β-Thalassemia trait in tribal population of Hingoli district; Maharashtra from July 2014 to May 2016. The study was conducted at IIMSR,Medical College,Jalna. In this study we screened 1075 tribal subjects comprising adult men & women as wellas children. Whole blood Samples were collected in EDTA bulb for Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT). The screening of â thalassemia trait was done on NESTROFT with 0.36% freshly prepared saline. Out of 1475 tribal subjects the NESTROFT was positive for 111 subjects which indicated very high chances of subjects having β-Thalassemia trait. With this rate of β thalassemia trait, urges necessity in studying beta thalassemia carrier status in the child bearing group, as a primary step to prevent the birth of beta thalassemia major.

Keywords: Tribal; β-Thalassemia Trait; NESRTROFT.

Indroduction

β- thalassemia is one of the most common single gene disorders in India with an overall prevalence of 3-4% [1]. In certain communities like Sindhis, Muslims, Cutchi Bhanushalis, and some tribal groups, the prevalence of β thalassemia carriers varies between 8 and 10% or more [2,3].

Reportedly, there are about 240 million carriers of â-thalassemia worldwide and in India alone the number is approximately 30 million with a mean prevalence of 3.3% [4,5]. It has been estimated that about 10 000- 12 000 children with â thalassemia major are born every year in India. These figures might be underestimated. As the frequency of thalassemia is increased by the consanguinity and endogamous mating, it may be assumed that the Sindhi communities in India are facing the problem at large scale. Three classes of β-thalassemia have long been recognized clinically, β-thalassemia major, intermedia and minor[6]**.** β- Homozygous state presents with variable degree of anemia from early childhood and are generally transfusion dependent, a condition clinically known as thalassemia major. β-heterozygous cases (thalassemic minor) are almost asymptomatic with normal or slightly reduced levels of hemoglobin. However an intermediate condition which may have either heterozygous or homozygous pattern of inheritance, requires minimal or no blood transfusion and has milder clinical course than thalassemic major but is severe enough as compared to thalassemic minor. It manifests generally after two years of age and does not require regular transfusion therapy [7,8].

There is growing concern that thalassemia may become a very serious problem in the next 50 years, one that will burden the World's blood bank supplies and the health system in general. Therefore emphasis has shifted from treatment to prevention of birth of such children in future [9]. The most effective approach to reduce the burden on the society and to reduce the disease incidence is through

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implementation of a carrier screening programme, offering genetic counseling, prenatal diagnosis and selective termination of affected fetus [10]. Need for prevention of thalassemia is obvious due to high frequency of the condition, the great expenses and difficulties in providing optimal treatment for patient. Prevention would not only be a good public health practice, but would be cost effective, as the ratio of treatment to prevention is 4:1 as shown in the study from Israel [11].

Tribal population in India is not a homogenous group. Further, because of their isolated existence and endogamy over centuries, different tribal populations have distinctive genetic identities. With industrialization and availability of jobs in different areas of India many of these tribal populations have migrated from their homelands to cities in search of jobs. Although these migrations are relatively in small proportions (5-10%) but the absolute number of tribal persons living in big cities and industrialized areas of the country is substantial [12].

In context of the achievement of education and exposure to developing world, the Indian tribes are far from using the 'freedom in choice' which basically relies on the dissemination of accuracy in information and sharing that at social and familial level. Delicate and proper understanding of the very sensitive psycho-social determinants and aspiration of the tribal societies need to be considered before implementing any counselling effort pertaining to haemoglobinopathies in India.[13]

Tribal groups in Maharashtra have shown a prevalence of β-thalassemia trait of 1.6 to 5.6 % [14].

Tribal population comprises 35-40% of total hingoli population.Common tribes in hingoli district are andh, bhill and thoti mostly dwelling in small villages of district. Investigations like haemoglobin electrophoresis & HPLC are helpful in diagnosing beta thalassemia . However, these investigations are either expensive or time-consuming or cumbersome and often require sophisticated equipment. Hence, cannot be used as effective tools for population screening.

For screening purposes, a test which is inexpensive, requires a small amount of blood, does not require sophisticated equipment and can be applied on the population as a whole is preferred. These requirements are met by a modified osmotic fragility test "NESTROFT" (Naked Eye Single Tube Red Cell Osmotic Fragility Test), a test first described by Kattamis et al [15].

Aims & Objectives

1. To study the prevalence of β– thalassemia trait in tribal people of hingoli district.
2. To make tribal people aware of prevention and management of β- thalassemia in order to decrease the burden of morbidity and mortality associated with the disease.

Material & Methods

Study Design

Cross sectional study.

Study Period

July 2014 to May 2016.

Ethical Approval

The study was approved by the IIMSR,Jalna Institutional Ethical Committee and due permission was taken from civil surgeon office at Civil hospital hingoli.

Inclusion Criteria

All the tribal subjects in the age range of 3 to 35 years were included in this study. This study aimed at targeting children and young population.

Site of Sample Collection

OPD of Civil hospital at hingoli city & Rural hospitals at kalamnuri , salegaon , aundha which are talukas of Hingoli district. Sample were collected in four phases in the form of camps organized at civil & rural hospitals of kalamnuri,aundha & salegaon taluka by Dr.Raviraj Naik and Dr. Sarita Dakhure.

Storage of Sample

Sample collected were stored in 3ml plastic cuvetts in freezer compartment of refrigerator.

Site of Sample Study

Biochemistry laboratory IIMSR,Jalna .

Method

3 ml of blood sample was collected in EDTA bulb, and all samples were screened for beta thalassemia

trait by using NESTROFT with 0.36% buffered saline solution. 2 ml of the 0.36% buffered saline solution was taken in one tube (10 cm x 1 cm diameter) and 2 ml distilled water was taken in another tube. A drop of blood was added to each tube and they were left undisturbed for 1/2 an hour at room temperature. Both the tubes were then shaken and held in NESTROFT test kit stand having white background with thin black line drawn over it. The line was clearly visible through the contents of the tube containing distilled water. If the line was similarly visible through the contents of the tube with the buffered saline, the test was considered negative. If the line was not clearly visible, the test was considered positive. A positive test indicates lowered red cell osmotic fragility, suggestive of thalassemia trait, and confirmed by Hb A2 level >3.5% performed by HPLC.

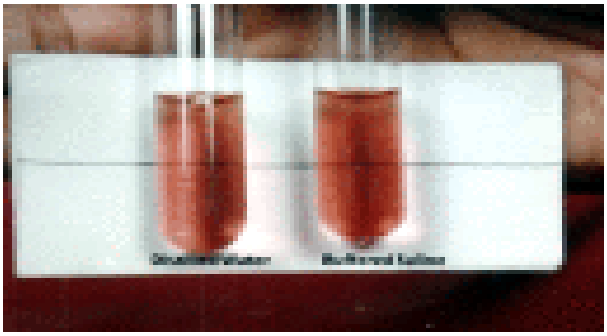

Fig. 1: Photograph showing negative NESTROFT

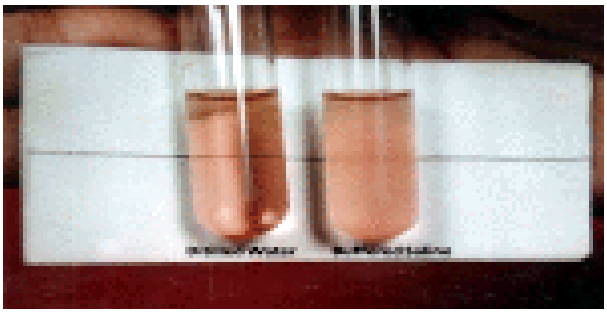

Fig. 2: Photograph showing positive NESTROFT

The tubes were left undisturbed for 3 hours. At the end of 3 hours, the DW tube was seen to be homogeneously pink with no sediments. In the BS tube the negative test showed similar findings as DW tube where as in a positive case, a clear supernatant and a sediment at bottom was observed [16].

Observation & Results

Blood samples of 1475 tribal people including men,women & children were taken for the study. The samples were subjected for NESTROFT test as they were available. After analyzing the data it was found that out of 1475 tribal people, 111 showed NESTROFT positive. So out of 1475 tribal subjects 47 men, 51 women and 13 children(8 boys & 5 girls) gave the NESTROFT test positive.

Table 1:

City/Taluka	Total Tribals Screened	Total tribals +ve for Nestroft test
Hingoli City	215	18
Kalamnuri taluka	566	37
Aundha taluka	445	29
Salegaon taluka	249	27
	1475	**111**

None of the tribal subjects which were positive for nestroft test were suffering from any clinical features of thalassemia; so they were labeled as silent carriers. Only 5 subjects were aware of the disease called as thalassemia and none of them were knowing about common occurrence of thalassemia in tribal population. Only 9 subjects among total screened were aware of the fact that consangious marriage should be avoided. All tribals arrived at camps at all 4 hospitals were councilled in simple and local language regarding prevention and management of thalassemia and also were advised all doand don't do in social life in order to decrease the burden of suffering from â thalassemia diasease in tribal society.

Those tribal subjects who gave nestroft test positive may be labeled as having β-thalassemia trait but nestroft test does not confirm the disease. Ideally subjects giving netsroft test positive should be further investigated by running their sample in Hb electrophoresis or HPLC which is best in this regard. Unfortunately we were not equipped with HPLC at our institute so samples were not further processed.We felt no need of sending the sample to other institute as none of nestroft positive subjects were symptomatic and severe anemic (Hb<7gm%).

Discussion

This study revealed that almost 7.52% of tribal population in hingoli district was suffering from β-thalassemia trait. Similar findings were reported by Sukumari et al [2] and Mehta et al [3]. However previous studies revealed that the tribal groups of India have high risk of beta-thalassemia, the prevalence of carrier status in some being as high as 17% [17]. Agarwal et al. [18] stated that the majority of the beta thalassemia carriers were of Uttar Pradesh origin. But according to Verma et al. [19], the majority of β-thalassemia carriers in India were migrants from Pakistan and their pattern of mutations differed from

the rest.

On the basis of earlier reports published since last 20 years, it is clear that several tribal groups of India have been identified as high-risk groups for thalassemia and other haemoglobinopathies. It causes high degree of morbidly and mortality among them [20]. In India, with about 4635 ethniccommunities five common and 12 rare mutations have already been reported [21,22]. Previous studies have given some probable causes of such high frequency of the disease among the tribal people. Migration of tribal groups from higher risk zone may be one of the causes of having high prevalence of [23,24] Similar haplotype for tribal groups from different parts of India may be consistent with the hypothesis of the Unicentric origin of the mutation in the globin chain as well as Unicentric origin of the tribal population.Due to the practice of non-random mating pattern for a long time, some particular mutations are restricted to some specific groups [25]. High inbreeding rate due to consanguineous practices of marriage make this situation more complex, because consanguinity is an important issue to spread the disease [26]. In addition to this, lack of proper medical facilities, natural barriers like forest, ecological niches etc., poverty, illiteracy, poor sanitation, lack of safe drinking water, faith in traditional beliefs and taboos [27] have further compounded the complexity [28].

Despite of considerable advancement in management strategies of thalassemia in India, the problem still remains for tribal and isolated populations. Because implementation of technological advances to the realities of health care in developing countries is a challenge. The strategy that has been taken in India is sometimes problematic to implement. Thus the problems related to the tribal groups claim a special intervention strategy for prevention and control of thalassemia, which may be more feasible for the Indian tribes. A strategy model for controlling thalassemia among the tribes of India may include treatment and prevention. The only curative treatment available is bonemarrow transplantation and iron chelation, which is highly expensive and not easily affordable by a tribal family. Prevention and control of new thalassemic baby is, therefore, more important to reduce the prevalence of these diseases.Prevention can be done through increasing the awareness and testing at a mass level. But lack of awareness and indifferent attitude towards thalassemia is very common among the tribal people. They are ignorant about the medical, social and financial burden of the disease. Thus in this prevention program priorities should be given on the public awareness, which can be done through community education, awareness camp, awareness at school level and motivation of high risk group. For these, schools, college and different government sectors may play major role with active involvement of media. In remote areas where even the media is not accessible, NGOs can take the responsibilities. An ideal screening programme of thalassemia trait for tribal woulde consist of definitive strategies such as ; extended family screening (i.e. the testing of the relatives of thalassemia patients), as the firstdegree relatives of a thalassemic patient have “14% higher risk of having an affected child compared to the general population”. Second level of testing is the carrier screening of unmarried girls and boys of the tribal communities. Next strategy that can be adopted is the genetic counseling of married couple before pregnancy.

Another strategy for screening the target population may be the testing of pregnant women attending hospital / healthcare unit, after which the husband is asked to be tested if the wife is found to be a carrier. The later one is a cost effective strategy as it reduces the screening cost by 50%. This test should be mandate for each and every pregnant woman and should be free of cost for them. And if the result of the test is positive, then the only way is the selective termination of affected fetuses. Apart from this, it may be noted that only awareness is not enough for tribal communities. Constant monitoring is essential through community participation which is possible only when health setup comprising of PHC & Rural Hospital are fully bestowed with facilities required for investing haemoglobinopathies such as thallasemias.

Conclusion

1. Out of 1475 tribal people screened, 111 showed NESTROFT positive and may be labeled as having â-thalassemi trait.
2. Tribal people should be made aware of different hemoglobinpathies common in tribal population and should be councilled properly to give up the dangerous tradition of consangious marriages.
3. Much further study needs to be undertaken in order to investigate for other hemoglobinpathies which are common in tribal population.

Referrences

1. Sood SK, Madan N, Colah R, Sharma S, Apte SV (eds). Collaborative studies on thalassemia: Report

of ICMR task force study; Indian Council of Medical Research, New Delhi, 1993.

2. Sukumaran PK, Master HR. The distribution of abnormal hemoglobinms in India. In: Sanghvi LD, Balkrishnan V, Bhatia HM, Sukumaran PK, Undevia JV, editors. Human population genetics in India. Mumbai: Orient Longman Ltd.1974; p. 91-111.
3. Mehta BC, Dave VB, Joshi SR, Baxi AJ, Bhatia HM, Patel JC. Study of hematological and genetical characteristics of Cutchi Bhanushali community. Indian J Med Res. 1972; 60: 305-11.
4. Verma IC. Choudhary VP. & Jain. PK. "Prevention of thalassemia:A necessity in India". Indian J Pediatr. 1992; 59: 649-654.
5. Yagnik H. "Post counselling follow-up of Thalassemia in high risk communities". Indian Pediatrics. 1997; 34(12): 1115-8.
6. Thein SL. "Genetic insights into the clinical diversity of beta thalassemia". Br.J Haematol. 2004; 124(3): 264-74.
7. Rund D., Oron-K V., Filon D.,Goldfarb A., Rachmilewitz E., & Oppenheim A. "Genetic analysis of â-thalassemia intermedia in ISRAEL:Diversityof mechanism and unpredictability of phenotype. AmJHaematol. 1997; 54: 16-22.
8. Tyagi S, Kabra M., Tandon N., Saxena R., Pati H., & Choudhary V. "Clinico-Hematological Profile of thalassemia Intermedia patients". Int J. Hum Genet. 2003; 3(4): 51-258.
9. Mallik S.,Chatterjee C.,Mandal P.,Sardar J.,Ghosh P., & Manna N. "Expenditure to treat Thalassemia an Experience at a tertiary care hospital in India".Indian J. Publ Health. 2010; 39(1): 78-84.
10. Talsania S,Talsania N.,& Nayak H. "A cross sectional study of thalassemia in Ahmadabad City,Gujarat". Healthline. ISSN 2229-337X, 2011 Jan-Jun; 2(1).
11. Ginseberg G,Tulchinsky T, Filon D,Goldfarb A, Abramov & L,Rachmilevitz EA. "Cost benefits analysis of a national thalassemia prevention programme in Israel". J Med Screening. 1998; 5: 120-6.
12. Migration of tribal woman - Planning Commission. 2010. 2. Available from: http://www.planning commission.nic.in, accessed on April 24, 2015.
13. Dipika Mohanty & Kishalaya Das;Genetic counselling in tribals in India; Indian J Med Res. October 2011; 134; 561-571.
14. Rao VR, Gorakshakar AC. Sickle cell hemoglobin, â-thalassemia and G6PD deficiency in tribes of Maharashtra, India. Gene Geogr. 1990; 4: 131–13.
15. Kattamis C, Effremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting beta Thalassemia trait. J Med Genet. 1981; 18(4): 266–70.
16. Sen AK, Kaur M. A comparison of screening test for Beta Thalassemia Trait NESTROFT v/s MOFTI and confirmation of results by ion exchange open column chromatography. Ind J Haemat & Blood Transf. 1998; 16(1): 31–3.
17. Vaz FE, Thakur CB, Banerjee MK, Gangal SG. Distribution of beta-thalassemia mutations in the Indian population referred to a diagnostic center. Hemoglobin. 2000; 24: 181-194.
18. Agarwal S, Pradhan M, Gupta UR, Sarwai S, Agarwal SS. Geographic and ethnic distribution of betathalassemia mutations in Uttar Pradesh, India. Hemoglobin. 2000; 24: 89-97.
19. Verma IC, Saxena R, Thomas E, Jain PK. Regional distribution of β-thalassemia mutations in India.Hum Genet. 1997; 100: 19-113.
20. Balgir RS, Mishra RK, Murmu B. Clinical and hematological profile of hemoglobinopathies in two tribal communities of Sundargarh district in Orissa, India. Int. J. Hum. Genet. 2003; 3940: 209.
21. Varawalla NY., Old JM., Venkateshanz SR., Weatherall DJ. The spectrum of beta thalas-semia mutations on the Indian subcontinent the basis of prenatal diagnosis. Brit J Hematol. 1991; 78: 242-247.
22. Das SK, Talukder G. A review on the origin and spread of deleterious mutations of the globin chain in Indian population. Homo (Germany). 2001; 522: 93-109.
23. Balgir RS. Do tribal communities show an inverse relationship between sickle cell disorders and glucose-6-phosphate dehydrogenase deficiency in malaria endemic areas of Central-Eastern India? Homo. 2006a; 57: 163-176.
24. De M, Chakraborty G, Das SK, Bhattacharya DK, Talukder G. Molecular studies of haemoglobin E in tribal populations of Tripura. The Lancet. 1997; 349: 1294.
25. Agarwal S, Pradhan M, Gupta UR, Yadav RS, Agarwal SS. Structural hemoglobin variants: Mutation, Hematology and its application in prenatal diagnosis. 2001.
26. Saxena A., Shubha P. Thalassemia control by carrier screening: The Indian Scenario. Curr. Sc. 2002; 83: 291-295.
27. Balgir RS, Das BP, Murmu B.Blood groups,hemoglobinopathy and G-6PD deficiency investigations among fifteen major scheduled tribes of Orissa, India. Anthropologist. 2004; 6: 69-75.
28. Balgir RS. Scenario of haemoglobin variants in Central-East coast of India. Curr. Sc. 2006b; 90: 1651-1657.

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Original Article

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Process Integration for Purification of Alcohol Dehydrogenase and Invertase from Baker's Yeast (*Saccharomyces Cerevisiae*)

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Abstract

The present work focuses on the integration of aqueous two-phase extraction with membrane processes (ultrafiltration / microfiltration) for the concentration and purification of alcohol dehydrogenase and invertase from crude extract of baker's yeast (*Saccharomyces cerevisiae*). Integration was carried out in three different modes. Integration of microfiltration (using nanofibrous membrane) with aqueous two-phase extraction was found to be best compared to other modes which has resulted in 8.12 fold purification with 647.32 U / mg specific activity and 95% enzyme activity recovery in case of alcohol dehydrogenase and 14.09 fold purification with 54.52 U / mg specific activity and 92.41% enzyme activity recovery in case of invertase.

Keywords: Aqueous Two-Phase Extraction; Bioseparation; Process Integration; Purification; Ultrafiltration; Yeast; Enzymes.

Introduction

Recent advances in biotechnology have focused on downstream processing, which constitutes a major portion of the production costs. However, much research has not been carried out in this area. There is a strong demand for downstream processing methods, which increase the yield, while reducing the process time and capital expenditure [1]. Process integration, wherein two unit operations are combined into one in order to achieve specific goals, which are not effectively met by these unit operations when they employed alone, offers considerable potential benefit for the recovery and purification of biological products [2]. It could be integration of extraction with membrane processes or different membrane processes with each other for achieving desired selectivity and purity of the biomolecule. Several integrated approaches have been developed to optimize productivity and cost effectiveness of different bioprocesses [3-5]. For instance the feasibility of aqueous two-phase extraction and membrane processes was demonstrated for the separation and purification of proteins [6]. Microfiltration and ultrafiltration have been widely used as preferred methods for protein concentration, purification, buffer exchange and has effectively replaced size exclusion chromatography [1, 7-9]. In view of the high commercial potential of the enzymes, several attempts have been made to obtain a stable enzyme preparation suitable for commercial application.

In order to evaluate the efficacy of the process integration, the work was carried out for the downstream processing of alcohol dehydrogenase (ADH) and invertase from baker's yeast (*Saccharomyces cerevisiae*), which are the main commercially valuable enzymes present abundantly in yeast.

Alcohol dehydrogenase (E.C. No. 1.1.1.1) enzyme is widely used in biochemical, forensic science for

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estimating the concentration of primary alcohols, NAD^+, ethylene glycol, numerous aldehydes and enzymatic catalysis of organic solvents and also in biosensors. Many investigations have reported successful purification of the ADH enzyme by immobilized dye-metal expanded bed affinity chromatography and other methods from the same source with purification factors ranging from 6.5 to 8.8 and recoveries ranging from 40.7 to 93.5% [10-13].

Invertase (β-D-Fructofuronoside fructohydrolase; E.C. 3.2.1.26) is mainly used in the food industry (confectionery, syrups, condensed milk, infant foods and beverages). It is also used for the manufacture of artificial honey, plasticizing agents used in cosmetics, pharmaceutical and paper industries as well as enzyme electrodes for the detection of sucrose. Various researchers have developed several methods for the purification of invertase [14-18]. There are many reports available for the purification of invertase resulted with good degree of purification however, the recovery was low. For example, purification of soluble acid invertase of sugarcane (*Saccharum officinarum L.*) was reported using combinations of precipitation and different kinds of chromatographic methods obtained about 13 fold purification with a recovery of 35% [19]. Other report on invertase purification using precipitation followed by DEAE-column chromatography resulted in 5.8 fold purification with recovery of 3.2% [20]. However, almost all these methods of purification involve a number of steps, namely, precipitation, ion-exchange chromatography, gel filtration chromatography etc., and it is known that higher the number of steps higher is the loss of product yield [21].

ATPE has been employed for the primary recovery and partial purification of a variety of biological products, including proteins, genetic material, low molecular weight products, cells and cell organelles [22-23]. The main advantages of this technique include scaling up feasibility, process integration capability and biocompatibility. The separation involving membrane processing and aqueous two-phase extraction (ATPE) can be performed at ambient temperature with less energy consumption in comparison to other separation processes. Hence, the integration of aqueous two-phase extraction and membrane process (ultrafiltration / microfiltration) was attempted in the present study for the concentration and purification of alcohol dehydrogenase (ADH) and invertase from baker's yeast to achieve higher purity without losing recovery.

The majority of the commercially available microfiltration / ultrafiltration membranes are inherently non homogeneous (non-uniform in mass and thickness), which affects the operational performance. Nanofibrous microfiltration / ultrafiltration membranes offer unique properties for filtration and adsorption based separations including high specific surface area, good interconnectivity of pores and the potential to incorporate active chemistry on a nanoscale. Hence, nanofibrous microfiltration was attempted along with ATPE for the recovery of the enzymes. Different combinations of ATPE and membrane process were used for downstream processing of these enzymes.

Materials and Methods

Materials

Polyethylene glycol (PEG, mol. wt 6000 and 20,000), peroxidase, sucrose, o-dianisidine, glucose oxidase, glycerol and glucose were procured from Sigma Aldrich, MO, USA. Potassium phosphate salts (KH_2PO_4, K_2HPO_4), trisodium citrate ($C_6H_5O_7Na_3$), hydrochloric acid (HCl), sodium sulphate (Na_2SO_4) were from Merck, Mumbai, India. Nicotinamide adenine dinucleotide (NAD^+) from Himedia (India), Ammonium sulphate ($(NH_4\text{-})_2SO_4$), magnesium sulphate ($MgSO_4$), sodium phosphate (Na_2HPO_4, NaH_2PO_4), were purchased from Ranbaxy Chemicals Gurgaon, India. All the chemicals used were of analytical grade. Baker's yeast was procured from local super market. Ultrafiltration membranes (100 kDa) and PVDF microfiltration membranes (0.45 mm, 47 mm) were procured from Millipore, USA and Microfiltration nanofibrous membranes were obtained from National University of Singapore (NUS).

Methods

Crude Extract Preparation

Crude extract was prepared using Baker's yeast (dry form, 1:10 ratio (w / v)) in 10 mM sodium phosphate buffer for ADH and in Tris buffer (50 mM, pH 7.5) for invertase extraction (8, 24). Disruption of yeast suspension was carried out using homogenizer (Ika, labortechnik, India) for 10 minutes at 10,000 rpm. The homogenate was centrifuged (CPR-24, Remi, India) for about 10 minutes at 10,000 rpm and the clear supernatant obtained (crude extract) was used for the experiments.

Protein Concentration

Bradford method [25] was used to determine the concentration of the protein using coomassie brilliant

blue G-250 dye as a reagent and bovine serum albumin (BSA) as standard, by measuring the absorbance at 595 nm at 25 °C in UV Spectrophotometer (Spectronic UV-160A, Shimadzu, Japan).

Enzyme Activity

ADH assay was carried out using ethanol as substrate in presence of NAD^+ (26). The absorbance, measured for 5 min at 340 nm, indicated the generation of NADH. One unit of ADH activity is defined as the amount of enzyme required for catalyzing the formation of 1.0 mmole acetaldehyde from ethanol per minute, at pH 8.8 at 25°C [27].

Invertase activity was measured as per the protocol described earlier [24] using sucrose as substrate and glucose was determined by glucose oxidase method One unit of invertase activity is defined as the amount of enzyme at pH 4.9 which hydrolyzes sucrose to produce 1 µmole of glucose / minute at 30^0 C [24].

Aqueous Two-Phase Extraction

Aqueous two-phase extraction experiments were carried out in the following manner. Predetermined quantities of polymers and salts from the phase diagrams [22, 28] were weighed and added to crude enzyme extract making the total weight of the system 100% on w / w basis. The contents were mixed thoroughly for 1 hour using a magnetic stirrer and were allowed to separate for about 8 hours in a separating funnel to obtain clear phase separation. The top and bottom phases were collected, volumes were noted and analyzed for protein and enzyme activity. An average of three replicates was considered. The error in the analysis was within ±2%.

Membrane Processes

Microfiltration and ultrafiltration were carried out using stirred cell module (Amicon solvent resistant stirred cell module, Millipore, USA; capacity of 50 ml). 100 kDa membrane disc of 47 mm diameter (Millipore, USA) was used for ultrafiltration. The nanofibrous membrane sheet (obtained from NUS, Singapore) was cut into discs of 47 mm diameter and PVDF membrane of 47 mm diameter (Millipore, USA) were used for microfiltration. The filtration experiments were carried out for 2h by maintaining the pressure, stirring speed and temperature constant throughout the experiment at 1.5 bar, 250 rpm and 25 ± 2 ºC, respectively. The pressure was applied using N_2 gas. Transmembrane flux was calculated based on the average flux.

Results and Discussion

Purification of alcohol dehydrogenase and invertase was carried out separately by employing three different modes (presented in Figure 1) from the crude extract of baker's yeast namely, Mode 1: ATPE followed by UF, Mode 2: UF (in diafiltration mode) followed by ATPE and Mode 3: MF followed by ATPE. ATPE experiments were carried out by selecting the standardized phase compositions from literature [8, 24].

Purification of ADH

ATPE Followed by UF (Mode 1)

Aqueous two-phase extraction of ADH was carried out employing PEG-20000 / potassium phosphate system (12 / 7.33 %, w / w). The top and bottom phases were separated after ATPE and the volumes were measured. The results are given in the Table 1. From the table it can be seen that ADH preferentially partitioned to the bottom phase with 96.94 % enzyme activity recovery. From ATPE alone 6.6 fold purification was obtained with specific activity of 522.62 U / mg. Further, the bottom phase was subjected to UF. Equipment used for membrane processing is shown in Figure 2. Ultrafiltration has shown some improvement in the purification factor (6.6 to 7.32) along with the removal of phase components and at the same time recovery was slightly reduced from 96.94 to 91.38 %.

UF Followed by ATPE (Mode 2)

The crude extract (50 ml) was subjected to ultrafiltration for 2 hr at 1.5 bar pressure using 100 kDa membrane to obtain 45 ml of permeate. Fig. 3 shows the comparison of flux rates in case of UF in normal mode and UF in diafiltration mode. Diafiltration mode was used to reduce both concentration polarization and membrane fouling in order to maintain the flux. It can be seen from the figure that UF in diafiltration has shown higher flux compared to UF (in normal mode). Around 20% of the contaminant proteins were removed from the crude extract during ultrafiltration (diafiltration mode) resulting in 1.24 fold enrichment of the enzyme. The retentate obtained was subjected to ATPE for further purification.

After ATPE, around 7.53 fold purification of ADH was observed with 97.4 % activity recovery in the bottom phase. Specific activity has enhanced to 600.29 U / mg compared to crude extract of 79.67 U / mg (Table 1).

MF followed by ATPE (Mode 3)

In this mode microfiltration was employed followed by ATPE for the purification of ADH. MF, using membrane with MWCO 0.45 mm, was employed for the clarification of crude extract which has resulted in around 1.3 fold enrichment. However, the recovery was slightly low (90%). MF was also carried out using nanofibrous MF membranes. Figure 4 shows the flux rates of normal PVDF and nanofibrous membranes and it can seen that nanofibrous membranes have resulted in higher flux (19 L / m^2h) with high recovery (96 %) compared to conventional PVDF membranes (6.5 L / m^2h). The permeate obtained was subjected to ATPE for further purification. After ATPE, 8.12 fold purification of ADH was observed in the bottom phase. In this mode, highest specific activity of 647.32 U / mg was obtained with 95 % activity recovery which is the best among the studied combinations.

Purification of Invertase

ATPE followed by UF (Mode 1)

ATPE was carried out at standardized conditions employing PEG-3350 / magnesium sulphate system (14 / 15, % w / w) for downstream processing of invertase [24]. The top and bottom phases were separated after ATPE and measured the phase volumes. The results obtained are given in the Table 1. From the table it can be seen that invertase preferentially partitioned to the bottom phase and resulted in 8.5 fold purification with 86.75 % enzyme activity recovery. The bottom phase was subjected to UF which has enhanced the purification factor from 8.5 to 10.89 fold, at the same time with a slight reduction in enzyme activity recovery (86.7 to 80.3%).

UF followed by ATPE (Mode 2)

Ultrafiltration of crude extract was carried out employing 100 kDa membrane. The system used for membrane process is same as shown in Figure 2. UF in diafiltration mode was used to for the processes because, UF in normal mode has shown lower permeate flux (4 L / m^2h at 120 min) compared to UF in diafiltration (7 L / m^2h at 120 min) (Figure 5). UF has shown removal of around 30% other low molecular weight contaminant proteins from the crude extract resulting in increased purification of 1.96 fold (Table 1). The retentate obtained was subjected to ATPE for further purification.

Table 1: Integrated approach for the purification of alcohol dehydrogenase

Mode of	operation	Phase	Protein Concentration (μg/ml)	Enzyme activity (U/ml)	Enzyme Specific activity (U/mg)	Degree of Purification (fold)	Enzyme activity Recovery (%)
	-	Crude	85.67	6.8	79.67	-	-
Mode 1	ATPE	Top	17.27	0.5	27.93	0.35	5.67
		Bottom	45.87	24.0	522.62	6.56	96.94
	ATPE-UF	Bottom	41.00	23.9	582.90	7.32	91.38
Mode 2	UF	Retentate	67.93	6.7	98.69	1.24	98.59
	UF-ATPE	Top	16.67	0.5	30.39	0.38	5.96
		Bottom	40.13	24.1	600.29	7.53	97.43
Mode 3	MF	Permeate	65.73	6.9	104.56	1.31	96.02
	MF- ATPE	Top	15.73	0.4	27.59	0.35	5.11
		Bottom	**39.27**	**25.4**	**647.32**	**8.12**	**95.32**

System: PEG-20000 / potassium phosphate (12 / 7.33 %, w / w)

Table 2: Integrated approaches for the purification of invertase.

operation	Phase	Protein Concentration (mg/ml)	Enzyme activity (U/ml)	Enzyme Specific activity (U/mg)	Degree of Purification (fold)	Enzyme activity Recovery (%)
-	Crude	2.87	11.1	3.87	-	-
ATPE	Top	2.55	5.0	1.94	0.50	17.86
	Bottom	0.88	28.9	32.94	8.51	86.75
UF	Bottom	0.63	26.8	42.16	10.89	80.34
UF	Retentate	1.60	12.1	7.59	1.96	94.76
UF-ATPE	Top	2.90	4.4	1.53	0.40	16.02
	Bottom	0.69	34.4	50.09	12.94	88.96
MF	Permeate	1.55	12.7	8.24	2.13	99.43
MF-ATPE	Top	2.86	5.0	1.76	0.46	18.17
	Bottom	**0.59**	**32.4**	**54.52**	**14.09**	**92.41**

System: PEG 3350 / Magnesium sulphate (14 / 15 % w / w)

The top and bottom phases were separated after ATPE and measured the phase volumes. Around 12.94 fold purification of invertase was observed with 88.96 % enzyme activity recovery in the bottom phase. Specific activity has enhanced to 50.09 U / mg compared to crude extract of 3.87 U / mg (Table 1).

MF Followed by ATPE (Mode 3)

MF with nanofibrous membrane has resulted in higher transmembrane flux (21.1 L / m^{2}h at 60 min) compared to that of MF with conventional PVDF membranes (6.5 L / m^{2}h at 60 min) (Figure 6). Microfiltration has clarified the crude extract and removed some of the contaminants (solutes / proteins) resulting in 2.13 fold purification and enzyme activity recovery of 99.43 %. The permeate obtained was subjected to ATPE for further purification. After ATPE, highest invertase purification of 14.09 fold with 92.41% activity recovery was observed in bottom phase.

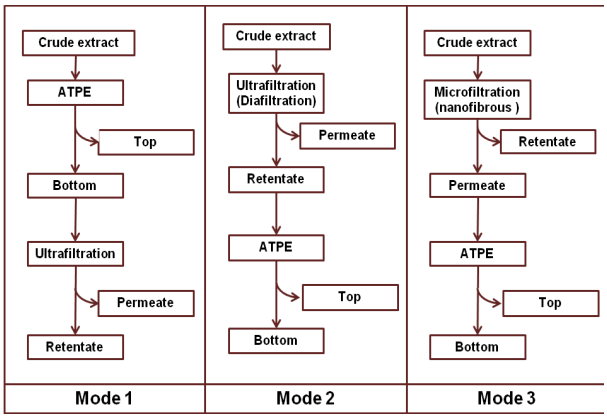


Fig. 1: Different combination of ATPE and membrane processes for downstream processing

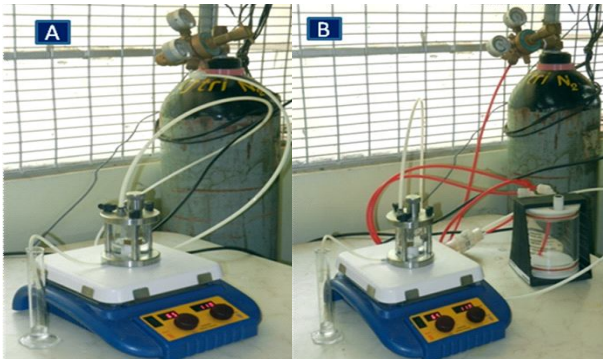
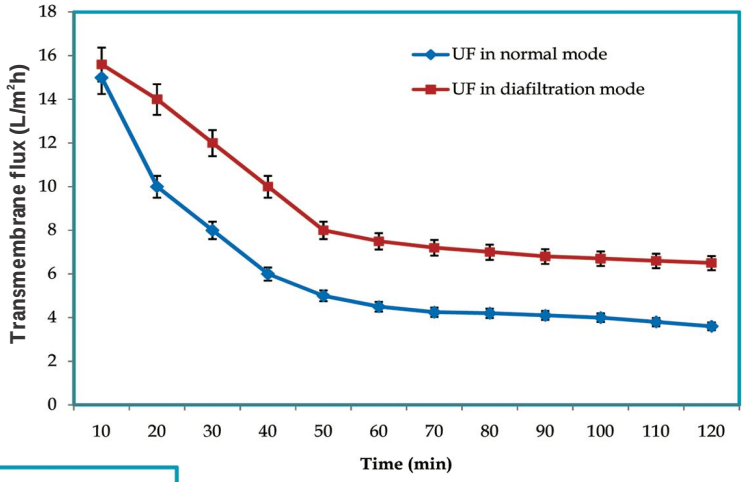


Fig. 2: Membrane processing set up used for the study. A) Ultrafiltration in normal mode; B) Ultrafiltration in diafiltration mode

Fig. 3: Transmembrane flux during ultrafiltration of ADH from crude yeast extract



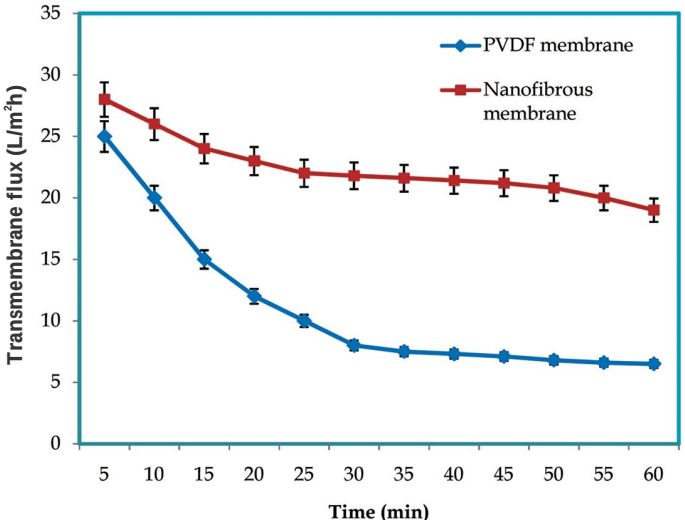


Fig. 4: Transmembrane flux during microfiltration of ADH from crude yeast extract

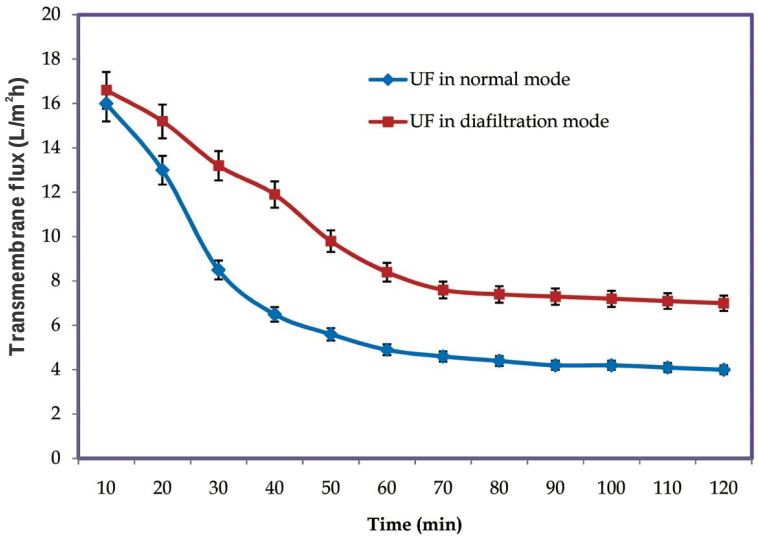


Fig. 5: Transmembrane flux during ultrafiltration of invertase from crude yeast extract

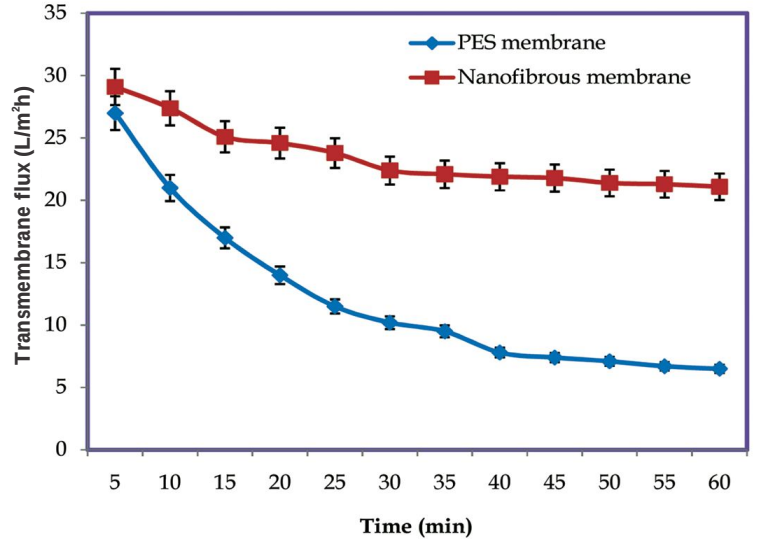


Fig. 6: Transmembrane flux dung microfiltration of invertase from crude yeast extract

Conclusions

The efficacy of integration of membrane processes with ATPE was demonstrated for the downstream processing of ADH and invertase from baker's yeast for achieving the higher degree of purification without losing of much yield. MF followed by ATPE was found to be the best among the combinations studied (3 modes) in case of both the enzymes. Integration of nanofibrous microfiltration with aqueous two-phase extraction has resulted in 8.12 fold purification with 95% activity recovery in case of ADH and 14.09 fold with 92.41% activity recovery in case of invertase.

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References

1. Schugerl, K.; and Hubbuch, J. Integrated bioprocesses. Curr. Opin. Microbiol. 2005; 8: 294-300.
2. Rito-Palomares, M. Practical application of Aqueous two-phase partition to process development for the recovery of biological products. J. Chrom. B. 2004; 807: 3-11.
3. Patil, G; Raghavarao, K.S.M.S. Integrated membrane process for the concentration of Anthocyanin. J. Food eng. 2007; 78: 1233-1239
4. Azevedoa, A.M., Rosaa, P.A.J.; Ferreiraa, I.F.; Aires-Barros, M.R. (2008) Integrated process for the purification of antibodies combining aqueous two-phase extraction, hydrophobic interaction chromatography and size-exclusion chromatography. *J. Chrom A* 1213: 154-161.
5. Tanuja, S.; Srinivas, N.D.; Gowthaman, M.K.; Raghavarao, K.S.M.S. Aqueous two-phase extraction coupled with ultrafiltration for purification of amyloglucosidase. Bioproc. Eng. 2000; 23: 63-68.
6. Srinivas, N.D.; Barhate, R.S.; Raghavarao, K.S.M.S. Aqueous two-phase extraction in combination with ultrafiltration for downstream processing of Ipomoea peroxidase. J. Food Eng. 2002; 54: 1–6.
7. Cheang, B.; Zydney, A.L. A two-stage ultraûltration process for fractionation of whey protein isolate. J. Membrane Sci. 2004; 231: 159-163.
8. Madhusudhan, M.C.; Raghavarao, K.S.M.S.; Nene, S. Integrated process for extraction and purification of alcohol dehydrogenase from Baker's yeast

involving precipitation and aqueous two-phase extraction. Biochem. Eng J. 2008; 38: 414-420.

9. Saxena, A.; Tripathi, B.P.; Kumar, M.; Shahi, V.K. Membrane-based techniques for the separation and purification of proteins: An overview. Adv. Colloid Interfac. 2009; 145: 1-22
10. Willoughby, N.A.; Kirschner, T.; Smith, M.P.; Hjorth, R.; Titchener-Hooker, N.J. Immobilized metal ion affinity chromatography purification of alcohol dehydrogenase from baker's yeast using an expanded bed adsorption system. J. Chrom. A. 1999; 840: 195–204.
11. Smith, M.P.; Bulmer, M.A.; Hjorth, R.; ; Titchener-Hooker, N.J. Hydrophobic interaction ligand selection and scale-up of an expanded bed separation of an intracellular enzyme from Saccharomyces cerevisiae. J. Chrom. *A.* 2002; 968: 121–128.
12. Hidayat, C.; Nakajima, M.; Takagi, M.; Yoshida, T. Development of new dye-metal agarose coated alumina matrix and elution strategy for purification of alcohol dehydrogenase. J. Biosci. Bioeng. 2003; 95: 133–138.
13. Hidayat, C.; Takagi, M.; Yoshida T. Expanded bed adsorption for purification of alcohol dehydrogenase using a dye-iminodiacetic acid matrix. J. Biosci. .Bioeng. 2004; 97: 284-287.
14. Gascon, S.; Neumann, P.N.; Oliver-Lampen, J. Comparative study of the properties of the purified Internal and external invertases from yeast. J. Biol. Chem. 1968; 243: 1573-1577.
15. Mislovicova, D.; Chudinova, M.; Gemeiner, P.; Docolomansky, P. (1995) Affinity chromatography of invertase on Concanavalin A-bead cellulose matrix: the case of an extraordinary strong binding Glycoenzyme. J. Chrom. B. 1999; 664: 145-153.
16. Chen, J.; Saxton, J.; Hemming, F.W.; Peberdy, J.F. Purification and partial characterization of the high and low molecular weight form (S- and F-form) of invertase secreted by Aspergillus nidulans. Biochim. Biophys. Acta . 1996; 1296: 207-218.
17. de-Almeida, A.C.S.; de-Araújo, L.C.; Costa, A.M.; de Abreu, C.A.M.; de-Andrade Lima, M.A.G.; de Los Angeles, M.; Palha, P.F. Sucrose hydrolysis catalyzed by auto-immobilized invertase into intact cells of Cladosporium cladosporioides. Electro. J. Biotechnol. 2005; 8: 55-62.
18. Basu, A.; Chaudhuri, P.; Malakar, D.; Ghosh, A.K. Co-purification of glucanase with acid trehalase–Invertase aggregate in Saccharomyces cerevisiae. Biotechnol. Lett. 2008; 30: 299–304.
19. Hussain, H.; Rashid, M.H.; Perveen, R.; Ashraf, M. Purification, kinetic and thermodynamic characterization of soluble acid invertase from sugarcane (Saccharum officinarum L.). Plant Physiol. Bioch. 2009; 47: 188–194.
20. Uma, C.; Gomathi, D.; Muthulakshmi, C.; Gopalakrishnan, V.K. Production, purification and characterization of invertase by Aspergillus flavus using fruit peel waste as substrate. Adv. Biological Res. 2010; 4: 31-36.
21. Kula, M.R.; Kroner, K.H.; Hustedt, H. Purification of enzymes by liquid–liquid extraction. Adv. Biochem. Eng. 1982; 24: 73–118.
22. Albertsson, P.A. Partition of cell particles and macromolecules, 3rd ed. Wiley Interscience, New York.1986.
23. Raghavarao, K.S.M.S.; Rastogi, N.K.; Gowthaman M.K.; Karanth, N.G. Aqueous two-phase extraction for downstream processing of enzymes / proteins. Adv. Microbiol. 1995; 41: 97-171.
24. Madhusudhan, M.C.; Raghavarao, K.S.M.S. Downstream processing of Invertase from baker's yeast: Effect of process parameters on partitioning. Process Biochem. 2011; 46: 2014-2020.
25. Bradford, M.A. Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. 1976; 72: 248-254.
26. Kagi, J.H.R., and Vallee, B.L. The Role of Zinc in Alcohol Dehydrogenase-V. The effect of metal-binding agents on the structure of the yeast alcohol dehydrogenase molecule. J. Biol. Chem. 1960; 235: 3188-3192.
27. Bergmeyer, H.U. Methods of enzymatic analysis, 1974; 1: 428-429. Academic Press, Inc., New York.
28. Zaslavasky, B.Y.Aqueous Two-phase Partitioning: Physical Chemistry and Bioanalytical Applications, Marcel Decker, New York. 1995.

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Original Article

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In Silico Screening of Bioactive Molecules From *Elephantopus Scaber* Linn. for Binding with Cardiac Potassium Ion Channels, Kir2.1 and Kir3.1

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Abstract

The earlier work in the lab has reported the identification of bioactive components from the methanolic extract of *E. scaber* Linn. These components are evaluated *in silico* for their cardiotonic properties and the binding energy is compared with positive controls; Dronaderone and azimilide, both class III anti-arrhythmic drugs. The ligand, 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine had exhibited efficient binding with Kir2.1 channel with a low binding energy of -10.14 kcal / mol; which was comparatively lower than Dronaderone (-9.06 kcal / mol). Similar result was obtained with the cardiac potassium ion channel, kir3.1, where the ligand ononin had better binding efficiency than the positive control, Azimilide with binding energies of -9.24 kcal / mol and -8.8 kcal / mol respectively. The present study has revealed 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine and ononin with better cardiotonic property that are devoid of arrhythmic side-effects.

Keywords: Cardiotonic Agents; Anti-Arrhythmic Drug; *Elephantopus Scaber;* Cardiac Potassium Ion Channels Kir2.1 and Kir3.1, Autodock 4, Lipinski Rule of Five.

Introduction

Cardiotonic drugs helps in maintaining a healthier condition for every tissue in the body through improved blood circulation via efficient heart muscle contraction. Cardiotonic drugs create a positive inotropic action (ie, elevated myocardial contraction) which in turn increases the blood flow through left ventricle resulting in an overall improvement of cardiac output. Cardiotonic drugs are mainly used in cases of heart failure, atrial fibrillation, atrial flutter and paroxysmal atrial tachycardia. The commonly used cardiotonics include cardiac glycosides or digitalis glycosides, but, their prolonged usage often leads to deleterious side effects like headache, weakness, drowsiness, visual disturbances, nausea, vomiting, anorexia and arrhythmias [1].

Arrhythmias defined as the irregular rhythm of heart is often characterized by abnormal faster rates (tachycardia) or slower rates (bradycardia) [2]. Arrhythmias arise due to genetic mutations of cardiac ion channels or their supporting proteins or alterations in their level of expression. The symptoms associated with arrhythmia include tiredness, difficulty in breathing, light headedness, dizziness, fainting (syncope) and, occasionally, chest pain. However, asymptomatic conditions of arrhythmias are also reported in several patients [3].

Anti-arrhythmic agents treat cardiac arrhythmias by blocking cardiac ion channels like sodium, potassium, calcium or the adrenergic receptors. Class III anti-arrhythmic agents like azimilide, dofetilide, dronedarone, ibutilide, sotalol, terikalant, amiodarone and bretylium block cardiac potassium

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channels, by lengethening refractory period. But, continous usage leads to side effects like torsade de pointe, pro-arrhythmia, pulmonary fibrosis, hypothyroidism, Optic neuritis leading to blindness, fatigue etc. [4].

Medicinal plants have been emphasized since time immemorial as cardiotonic agents. Digitalis, a common cardiotonic drug has been isolated from the leaves of *Digitalis purpurea* and *Digitalis lanata* [1]. Several plants have been reported to possess cardiotonic property like *Sarothamnus scoparius, Fumaria officinalis, Zea mays, Crataegus monogyna* etc.. (http://www.botanical-online.com/remediesheartfailure.html). *Elephantopus scaber* too has been reported to be a cardiotonic agent [5]. In the present study, the cardiotonic property with emphasis on its anti-arrhythmic nature, of bioactive molecules identified from *E. scaber* is evaluated *in silico* by docking it with cardiac inward rectifier potassium channel, Kir2.1 and subunit of acetylcholine activated inward rectifier potassium channel, Kir3.1; as protein targets and were compared with positive controls dronedarone and Azimilide, both Class III antiarrhythmic drugs.

Materials and Methods

Softwares

Protein Data Bank (http://www.rcsb.org/pdb/); ChemSpider database (http://www.chemspider.com/); ChemSketch from Advanced Chemistry Development, Inc. (ACD/Labs); Molsoft online molecular property calculator http://molsoft.com/mprop/; Open Babel software version 2.3.2. (http://openbabel.org/ (accessed 20.03.2015)); Swiss-Pdb Viewer version 4.1.0 (http://www.expasy.org/spdbv/); Auto Dock 4.0 (http://autodock.scripps.edu/downloads); Cygwin64 Terminal (http://www.cygwin.com/); Discovery Studio 4.1 Client (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php/)

Ligand Preparation

Eleven molecules identified from the methanolic extract of *E. scaber* were selected as ligands for the study which included Methylumbelliferone, Hydroxydihydrobovolide, Lysine theophylline, Ononin, Alismorientol A, Lotaustralin, 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine, Phytosphingosine, Chamazulene, Ethyl oleate and Piperine [6]. The structure of positive control, azimilide and dronedarone, were identified from ChemSpider database (http://www.chemspider.com/) and 3D structures of all these ligands were built with ChemSketch software developed by Advanced Chemistry Development, Inc. (ACD/Labs) and were subsequently converted to pdb format using the Open Babel software (Open Babel, version 2.3.2, http://openbabel.org (accessed 20.03.2015) for virtual screening.

Determination of Physico-Chemical Properties of the Ligands

The determination of molecular properties and drug likeness of the ligands is an important parameter to be looked upon and was determined using http://molsoft.com/mprop/. The percentage of absorption was calculated using equation: % ABS = 109 – (0.345 × TPSA) according to Zhao *et al* [7].

Preparation of Protein Structure

The protein crystal structure of cytoplasmic domains of the inward rectifier potassium channel, Kir2.1 (PDB ID: 1U4F) [8] and the acetylcholine-activated potassium channel, Kir3.1 (PDB ID: 1U4E) [8] was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/) and subjected to protein optimization via removing all heteroatoms and energy minimized with Swiss-Pdb Viewer version 4.1.0 (http://www.expasy.org/spdbv/) [9].

Docking

The receptor-ligand interaction was performed with Autodock 4.0 which consists of two programs termed Autogrid and Autodock. Autogrid precalculates grid parameters like center of the grid, 3D search space and spacing between points whereas the latter performs docking of the molecules to the receptor, defined by these set of grids. A default grid spacing of 0.375 Å with its grid points set to 60 Å each in X, Y and Z coordinates was created with AutoDock 4.0 to analyze ligand-receptor interaction [10]. For Kir2.1, the grid box was centered on 42.305, 27.455 and 36.223 while, Kir3.1 was centered on 9.969, 38.489 and 27.193 based on the x,y and z coordinates of amino acid residues, Glu224 and Asp260 present on the active site of two proteins respectively [11]. The search was based on the Lamarckian genetic algorithm.

For each ligand, a docking experiment consisting of 10 simulations was performed using Cygwin64 Terminal and the analysis was based on binding free energies and the ligand molecules were then

ranked in the order of increasing docking efficiency. The outputs were then exported to Discovery Studio 4.1 Client for visual inspection of the binding modes and interactions of the ligands with amino acid residues in the active sites.

Results

Molecular Properties and Drug Likeness of the Ligands

In the present study 11 bioactive molecules from *Elephantopus scaber* were screened with cardiac potassium ion channels, Kir2.1 and Kir3.1 taken as specific protein targets. The ligand molecules were analyzed for its molecular properties, drug likeness and percentage of absorption, prior to docking (Table 1). All the ligands except lysine theophylline (HBD > 5) and ethyl oleate (Mollogp > 5) were found to follow Lipinski's rule of five. The drug likeness score of the ligand molecules were comparable with the positive controls Dronedarone and Azimilide.

Lipinski's rule of five helps in the selection of molecules that could be developed as drugs. The ligand molecules following Lipinski's rule is reported to have theoretically better absorption, permeability and oral bioavailability [12]. The rule states that the molecular weight (MW) of the ligand should be ≤ 500, the Hydrogen Bond Acceptor (HBA) and Donor (HBD) groups in the ligand should be ≤ 10 and 5 respectively and mol log P value; which is the partition coefficient of the component in water: octan-1-ol system ≤ 5. The hydrogen bonding between the ligand and the receptor is a vital factor determining drug permeability. The strong binding often results in poor absorption and poor permeability. The mol log P and mol log S value represents the lipophilicity and aqueous solubility of the ligands. Lipophilic drugs can be easily taken up by the surrounding tissues from gastro-intestinal tract whereas solubility determines the uptake of drug by blood from the site of administration.

Other parameters like Topological Polar Surfce Area (TPSA), % absorption, No: of stereo-centres and drug likeness score are also considered. TPSA defined as the sum of surfaces of polar atoms in a molecule, predicts the drug transport properties and cell permeation properties. The recommended TPSA values are < 140 A^0 and 90 A^0 for cell permeation and blood-brain barrier respectively. The percentage of absorption is calculated from TPSA value using equation: % ABS = 109 – (0.345 × TPSA). It is observed that with increase in TPSA value, the percentage of absorption was found to be decreased. Less no: of stereo-centres suggest that upon binding, the ligand undergoes only a slight conformational change. The drug likeness is a qualitative concept which predicts the likeness of a molecule to a drug. Overall, the bioactive molecules of *E. Scaber* exhibited the presence of relevant pharmacophoric groups in them.

Table 1: Physico-chemical properties of the ligands

S no:	Component	MW	HBA	HBD	MlogP	MLogS	MV	N-SC	DL	TPSA (A^0)^2	% ABS
1	Methylumbelliferone	176.05	3	1	1.81	-2.60	192.40	0	-0.43	38.26	95.8003
2	Hydroxyl dihydrobovolide	198.13	3	1	2.40	-1.36	246.39	1	-0.61	39.03	95.5347
3	Lysine theophylline	326.17	7	6	-2.80	-1.28	316.45	1	0.73	124.67	65.9889
4	Ononin	430.13	9	4	0.70	-4.54	403.49	5	-0.02	108.26	71.6503
5	Alismorientol A	272.20	4	4	1.61	-0.80	327.66	6	-0.59	62.21	87.5376
6	Lotaustralin	261.12	7	4	-1.94	-0.99	256.22	6	-0.12	96.40	75.742
7	2-amino-4-(4-phenylpiperazino)-1,3,5-triazine	256.14	3	2	1.59	-1.80	228.68	0	-0.22	57.45	89.1798
8	Phytosphingosine	317.29	4	5	3.51	-5.35	353.16	3	-1.59	70.25	84.7638
9	Chamazulene	184.13	0	0	4.76	-5.01	222.28	0	-1.16	0.00	109.00
10	Ethyl oleate	310.29	2	0	7.98	-6.67	388.60	0	-0.78	20.67	101.8689
11	Piperine	285.14	3	0	3.96	-4.88	328.92	0	-0.02	33.47	97.4529
12	Dronedarone	556.30	6	1	7.72	-10.46	578.80	0	1.02	74.61	83.2595
13	Azimilide	457.19	6	0	3.22	-3.27	468.51	0	1.72	58.24	88.9072

Receptor-Ligand Interaction

In the virtual screening, inhibition activity of the bioactive molecules towards the target proteins was analyzed by comparing with Dronedarone and Azimilide, known inhibitors of Kir2.1 and Kir3.1 respectively. The best possible binding modes of the bioactive molecules at the targeted protein's active sites were observed using Discovery Studio 4.1 Client and corresponding energy values were recorded.

Binding affinities of plant molecules were analyzed and ranked according to lower energies (Table 2). The interaction of components identified

Table 2: Binding energies of bioactive molecules from *Elephantopus scaber* towards the cardiac potassium ion channel, Kir2.1 and Kir3.1

Sl. No	Bioactive molecules	During ligand-Kir2.1 interaction.		During ligand-Kir3.1 interaction	
		Binding energy (kcal/mol)	Key protein ligands interaction	Binding energy (kcal/mol)	Key protein ligands interaction
Std	Dronedarone / azimilide	-9.06	ARG260, ASP259, MET301, ARG260, GLU224, ALA306, HIS226	-8.8	THR210, HIS222, THR257, ARG229, HIS272, ASP260, CYS271, ALA259
1	2-amino-4-(4-phenylpiperazino) -1,3,5-triazine	-10.14	GLU224, GLY300	-7.66	SER256, ASP275, VAL273, ASP252
2	Ononin	-8.55	HIS226, ARG260, ARG228,ALA225, GLU224, HIS226, GLU299, HIS226, ARG260, ALA306, HIS226	-9.24	GLN227, VAL253, SER256, THR257,PHE263, CYS271, VAL273, LEU251, ILE228, ASP260, GLN261, ARG229, ASP252, ALA259, GLU250, CYS271, VAL273, GLN227, GLN261
3	Hydroxydihydrobovolide	-8.13	ARG228,GLN230, ARG260,PHE262, HIS226, GLU299, ALA225, ALA306	-7.93	VAL253, PHE255, VAL273, ASP252
4	Lotaustralin	-7.05	GLN310,MET301, GLU224,THR308, GLN310, THR309	-7.61	HIS222,ALA226, GLN227,VAL253, SER256, THR257, VAL273, LEU262, ILE228, LEU251, ARG229, PHE263, PHE255
5	Phytosphingosine	-6.9	ASP259, ALA306, HIS226	-8.57	SER256, ALA259
6	Ethyl oleate	-6.88	ARG260,GLU299, ARG260,GLU299, ALA306, ALA225	-7.91	ASP252, VAL273, GLU250,VAL253, HIS272, PRO279, VAL273
7	Piperine	-6.72	GLU224	-8.0	PHE263,CYS271,VAL273, ASP252, ILE270, VAL253, CYS271, VAL273, ALA259
8	Alismorientol A	-6.57	ARG260,ARG312, HIS226, ARG260, THR309, HIS226	-7.27	VAL253, LEU251, ASP252, GLU250
9	Methylumbelliferone	-6.06	ARG312,ALA304, THR308,GLN310, ALA306, THR309	-5.94	VAL253, HIS272, VAL273, ILE228, ASP260, HIS272
10	Chamazulene	-5.5	GLU299, GLN310	-5.86	-
11	Lysine theophylline	-3.92	ARG228,ARG312, HIS226, ALA304, GLU224,THR308, GLN310,GLU303, THR309,GLU303, ALA225,GLN310	-3.54	VAL253,GLU250, ASP252, ILE228, ASP260, GLU250, PHE255

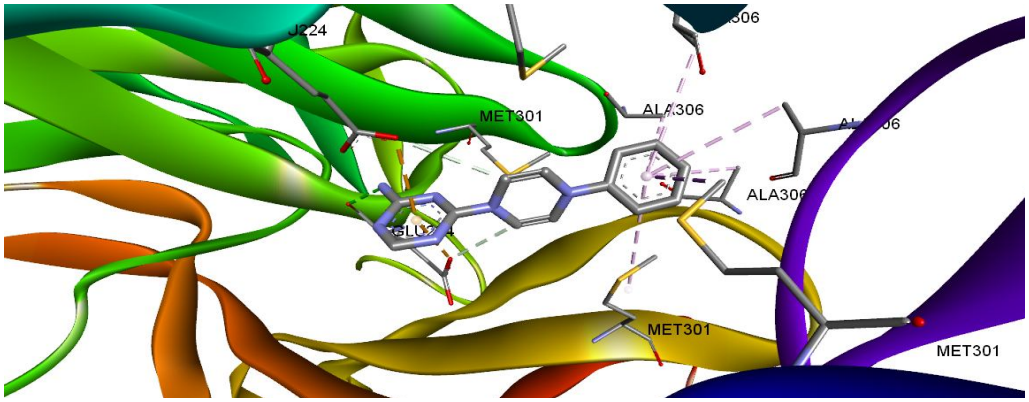

Fig. 1(A): The docked pose of 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine at the active site of cardiac inward rectifier potassium ion channel Kir2.1, 1U4F. The key amino acids interacting with the ligand is labeled in the figure

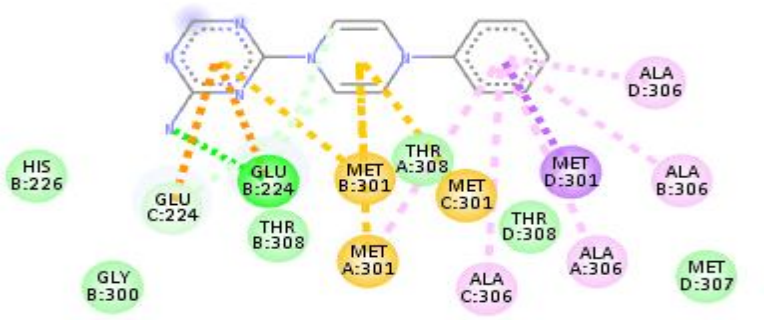

Fig. 1(B): Represents the protein-ligand interactions. Different bindings are shown in various colors. : Van der waals interaction, : Conventional hydrogen bond, : Carbon hydrogen bond, Pi- alkyl interaction, Pi-Sulfur, Pi-Antion and : Pi-Sigma.

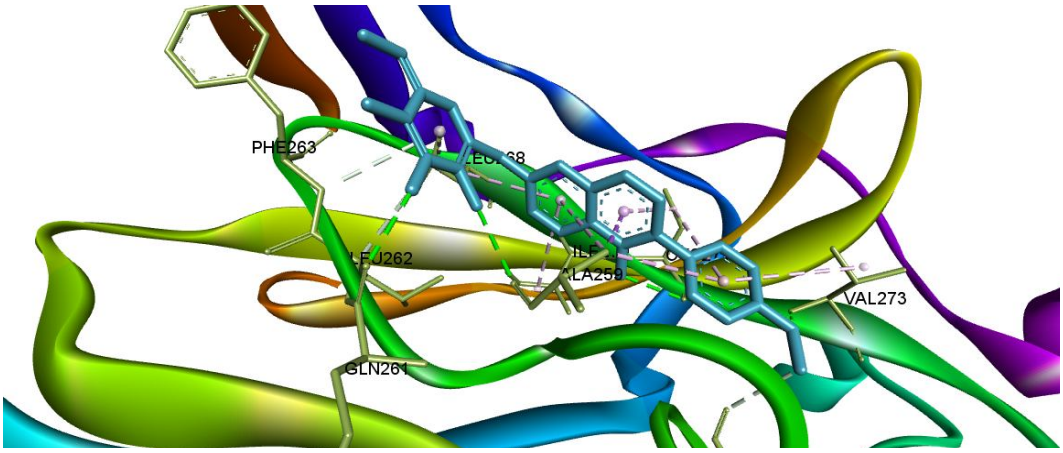

Fig. 2(A): The docked pose of ononin at the active site of G-protein-coupled inward rectifier potassium ion channel Kir3.1, 1U4E. The key amino acids interacting with the ligand is labeled in the figure

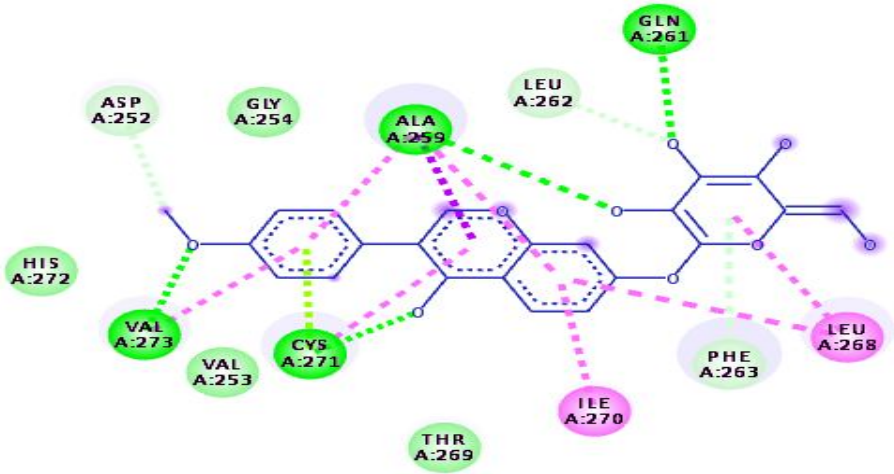

Fig. 2(B): Represents the protein-ligand interactions. Different bindings are shown in various colors. : Van der waals interaction, : Conventional hydrogen bond, : Carbon hydrogen bond, Pi- alkyl interaction, : Pi-Sigma, : Pi-Donor hydrogen bond and : Pi lone pair

from *E. scaber* with the cardiac potassium ion channel, kir2.1 revealed that 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine (Figure 1A & B) had exhibited excellent binding than the positive control, Dronedarone with binding energies of -10.14 kcal / mol and -9.06 kcal / mol respectively. The components, ononin and hydroxydihydrobovolide have comparable binding energies with the positive control, Dronedarone for interaction with Kir2.1 (-8.55 kcal / mol and -8.13 kcal / mol). Similar result was obtained with the cardiac potassium ion channel, kir3.1, where the ligand ononin (Figure 2A and B) had better binding

efficiency than the positive control, Azimilide with binding energies of -9.24 kcal / mol and -8.8 kcal / mol respectively. Phytosphingosine and piperine with binding energies of -8.57 kcal / mol and -8.0 kcal / mol too exhibited better binding with the cardiac potassium ion channel, kir3.1.

Discussion

Kir 2.1 and Kir 3.1, components of the family of inwardly rectifying potassium ion channels, play a significant role in maintaining cardiac resting membrane potential, shape and duration of cardiac action potential curve and membrane excitability [8]. The impaired conductance of ions through these cardiac ion channels often leads to irregular heartbeat, either too fast or too slow, termed Cardiac arrhythmia. Several inward rectifier potassium channel blockers like Amiodarone, Azimilide and Chloroquine (Kir2.1 blockers) as well as Dronedarone Disopyramide, Flecainide (Kir3.1 blockers) have been identified as novel therapeutic agents for cardiac arrhythmias. They basically bind to these cardiac ion channels and prevent ventricular fibrillation and shortening of Action Potential Duration (APD) leading to therapeutic implications on various pathological conditions manifested by ventricular arrhythmia like myocardial ischemia, coronary heart Disease etc. [13].

Along with many of its beneficial effects, anti-arrhythmic drugs possess serious side effects that may even rise to more complicated rhythm disorders than ones being treated. Therefore, the search for ligand molecules which have cardiotonic properties and are devoid of arrhythmia has been an area of investigation. Ligands identified from the methanolic extract of *E. scaber,* a plant known to possess cardiotonic properties was screened with optimized and energy minimized 1U4F and 1U4E**;** cardiac inward rectifier potassium channel, Kir2.1 and Kir3.1 respectively. The protein optimization and energy minimization brings down the energy of macromolecules to a lower level as seen in the native cellular environment; by reducing the sterric clashes and bringing in more orientations that are similar to the theoretical true binding mode.

Upon docking, 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine and ononin exhibited better binding efficiency than their corresponding positive controls. The binding of other ligands like Ononin, Hydroxydihydrobovolide and Lotaustralin (upon interaction with Kir 2.1) and phytosphingosine, piperine, hydroxydihydrobovolide and ethyl oleate (interaction with Kir 3.1) were comparable with positive controls. Binding energy is the amount of energy by which a ligand binds to the target protein. The lower the binding energy required by a ligand, greater will be its affinity towards the protein.

Prior to docking, the ligand molecules were analyzed for its molecular properties and violations of Lipinski rule of five. All ligands except lysine theophylline, (HBD > 5) and ethyl oleate (Mollogp > 5) were found to follow Lipinski's rule of five. The ligand molecules following Lipinski's rule is reported to have theoretically better absorption, permeability and oral bioavailability [12]. Topological Polar Surface Area (TPSA) values for the ligands were appropriate enough for efficient permeability through cellular plasma membrane. The value for water solubility (Mollog S) for the ligands ranged from -6.67 to -0.80. Overall, the ligands found in the bioactive fraction of *E. scaber* exhibited the presence of relevant pharmacophoric groups in them.

Arrhythmia, fluctuations in cardiac membrane potential is often due to the inflammations prevailing in the myocytes and interstitium (Chronic myocarditis). In fact, arrhythmia is reported to be the only clinical symptom in natural course of this disease. Myocardial inflammation which leads to micro- and macrovascular perfusion resulting in myocardial ischemia is reported to elevate the incidence of cardiac arrhythmogenicity [14]. The present study highlights its relevance, as bioactive molecules identified from *E. scaber*, reported to possess anti-inflammatory property [15] marked their efficiency as cardiotonic agents by inhibiting cardiac inward rectifier potassium ion channels Kir2.1 and Kir3.1 better than or comparable with the positive controls.

Conclusion

The present study has revealed the efficacy of 2-amino-4-(4-phenylpiperazino)-1,3,5-triazine, Ononin, Hydroxydihydrobovolide, Lotaustralin phytosphingosine, piperine, and ethyl oleate as cardiotonic agents that bind with cardiac potassium channels with high affinity. It was also clear, that these ligands exert cardiotonic property without posing much arrhythmic incidences. The physico-chemical properties of the ligands too indicate the presence of relevant pharmacophoric groups which can be further harnessed for drug development.

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References

1. Drugs, I. Cardiotonics and Miscellaneous Inotropic Drugs. Appl. Ther. Clin. Use Drugs 2008.
2. Amin, A. S.; Tan, H. L.; Wilde, A. A. M. Cardiac ion channels in health and disease. Heart Rhythm. 2010; 7: 117–26.
3. Li, G. R.; Dong, M. Revisit of the Cardiac Inward Rectifier Potassium Current I K1. 2010; 95–102.
4. Homoud, M. K. Introduction to Antiarrhythmic Agents Anti Arrhythmic Agents Introduction to Antiarrhythmic Agents. Spring; 2008; 1–21.
5. Amrita, B. Nephroprotective Activity of Ehanolic Extract of Elephantophus Scaber Leaves on Albio Rats. ISSN 2230 – 8407. 2012; 3: 246–250.
6. Abhimannue, A. P.; Mohan, M. C.; B, P. K. Inhibition of Tumor Necrosis Factor-α and Interleukin-1â Production in Lipopolysaccharide-Stimulated Monocytes by Methanolic Extract of Elephantopus scaber Linn and Identification of Bioactive Components. Appl. Biochem. Biotechnol. 2016.
7. Zhao, Y. H.; Abraham, M. H.; Le, J.; Hersey, A.; Luscombe, C. N.; Beck, G.; Sherborne, B.; Cooper, I. Rate-limited steps of human oral absorption and QSAR studies. Pharm. Res. 2002; 19: 1446–57.
8. Pegan, S.; Arrabit, C.; Zhou, W.; Kwiatkowski, W.; Collins, A.; Slesinger, P. A.; Choe, S. Cytoplasmic domain structures of Kir2.1 and Kir3.1 show sites for modulating gating and rectification. Nat. Neurosci. 2005; 8: 279–87.
9. Guex, N.; Peitsch, M. C. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis. 1997; 18: 2714–23.
10. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. NIH Public Access. 2010; 30: 2785–2791.
11. Noujaim, S. F.; Stuckey, J. A.; Ponce-balbuena, D.; Ferrer-villada, T.; Lo, A.; Pandit, S.; Calvo, C. J.; Grzeda, K. R.; Berenfeld, O.; Sa, A. Specific residues of the cytoplasmic domains of cardiac inward rectifier potassium channels are effective antifibrillatory targets.
12. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. 2001; 46: 3–26.
13. Tamargo, J.; Tamargo, J.; Caballero, R.; Caballero, R.; Gómez, R.; Gómez, R.; Valenzuela, C.; Valenzuela, C.; Delpón, E.; Delpón, E. Pharmacology of cardiac potassium channels. Cardiovasc. Res. 2004; 62: 9–33.
14. Klein, R. M.; Vester, E. G.; Brehm, M. U.; Dees, H.; Picard, F.; Niederacher, D.; Beckmann, M. W.; Strauer, B. E. [Inflammation of the myocardium as an arrhythmia trigger]. Zeitschrift fur Kardiol. 2000; 89 (Suppl 3): 24–35.
15. Kumar, B. P.; Abhimannue, A. P.; Mohan, M. C.; Jacob, J.; Babu, B. M. Research Article Inhibition of Lipoxygenase by Elephantopus Scaber Extractand Determication of its Inhibition Pattern. J. Adv. Sci. Res. 2015; 6: 1–5.

Original Article

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The Impact of Serum Uric Acid and Vitamin D on Essential Hypertension

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Abstract

Background: Hypertension is the third leading killer disease in the world and is responsible for 1 in every 8 deaths. Uric acid has been associated with hypertension in many studies involving different population but results were controversial and no information was found on the association between vitamin D insufficiency and elevated uric acid. The aim of present study was to assess the serum uric acid and vitamin D level in essential hypertension and try to find out its correlation. *Material and Methods:* The present study was case-control study. Total 90 subjects were included and divided into two groups. Group I consisted 45 subjects of essential hypertension in the age group 25-75 years while Group II consisted of age and sex matched 45 normal healthy individuals who served as control with no history of essential hypertension. Serum levels of uric acid, vitamin D were estimated in all the subjects under study. Values were expressed as mean± standard deviation. SYSTAT version 12 software was used for statistical analysis.Comparisons of study groups to control groups were done by applying student t test. Pearson's correlation coefficient was used to find out the correlation between two variables. *Results:* Serum uric acid level was increased significantly ($p<0.001$) in essential hypertension as compared with controls. Correlation between uric acid and diastolic blood pressure and systolic blood pressure was positively correlated and significant. Correlation between Vitamin D and diastolic and systolic blood pressure was negatively correlated and non-significant. *Conclusion:* In the present study,It can be concluded that, the essential hypertension is associated with abnormalities in the level of serum uric acid and vitamin D. Serum uric acid and vitamin D can be used as biochemical markers to determine severity of hypertension and it may be beneficial for better management and for developing new treatment strategies.

Keywords: Essential Hypertension; Uric Acid; Vitamin D.

Introduction

Hypertension is an important worldwide public-health problem because of its high frequency and concomitant risk of cardiovascular and kidney disease [1]. It is common in majority of readily detectable, usually treatable and often leads to lethal complications if left untreated [2].

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A recent report on the global burden of hypertension indicates that nearly 1 billion adults had hypertension in 2000 and this is predicted to increase to 1.56 billion by 2025. Hypertension is a major health burden and leading cause of death in the world. Although it is common in economically developed countries [3].

In India, awareness of hypertension and its complications is very poor. Poor awareness of normal blood pressure values in hypertension can be important factor hindering blood pressure control [4].

The pathogenesis of essential hypertension is not

clearly understood. Different investigators have proposed the kidney, the peripheral resistance vessels and sympathetic nervous system as the seat of primary abnormality [5].

The endothelial dysfunction could facilitate the maintenance of elevated peripheral resistance which could favors the occurrence of complication such as atherosclerosis, myocardial infarction and heart failure [6].

Uric acid is byproduct of purine metabolism produced in blood from endogenous purine substances or from diet. Alcoholic , high purine foods consumption low water consumption and poorly exercising are contributing factor responsible for hyperuricaemia [7].

Uric acid might be a cause of hypertension or renal disease. Uric acid plays an important role in hypertension mediated by several mechanisms such as inflammation, vascular smooth muscle cell proliferation in renal microcirculation and endothelial dysfunction [8].

Incidence of Hypertension in the general population rises with the increase in latitude which in turn is associated with low UV irradiation levels. Dark pigmentation in the black population which effects an efficient UV light penetration has been associated with a higher blood pressure [9].

Many studies in India have demonstrated that the level of VitaminD in the population is low and there is high prevalence of chronic disease like hypertension, diabetes, cardiovascular disease. Therefore in present study we examined serum uric acid andvitamin D level in patients with essential hypertension and their role in etiopathogenesis of essential hypertension.

Material and Method

The present study was conducted at Department of Biochemistry, PDVVPF's Medical College Ahmednagar. The study was approved byInstitutional Ethics Committee. All participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

Study Type

Case- Control study.

Study Design

Total 90 samples were enrolled in the present study.

Control Group

45 healthy age and sex matched individuals without any evidence of essential hypertensionas per Clinical examination by physicians in medicine OPD were taken as control subjects.

Patients Group

The study included total 45 patients between age group 25-75 years with essential hypertension.

Inclusion Criteria

a. Patients with essentialhypertension systolic blood pressure ≥140 and diastolic blood pressure ≥90 mm of Hg attending medicine Output patient department.
b. Controls are healthy individuals, age and sex matched without any major illness and not on any medication

Exclusion Criteria

Patients with secondary hypertension, complications of cardiovascular, renal disorders, and stroke, history of multiple transfusions, liver diseases, pregnancy, anemia and history of any other medical or surgical illness were excluded.

Method of Collection of Data

A pre-structured and pre-tested proforma was used to collect the data. Informed consent was taken from all cases and control subjects. Baseline data including age, sex, detailed medical history, clinical examinations and relevant investigations were included as part of methodology.

Collection of Blood Sample

About 5 ml of venous blood was drawn from subjects under aseptic precautions, using a sterile disposable syringe and collected in clot activator and fluoride EDTA vacuum evacuated tubes. After an hour, the samples were centrifuged at 3000 rpm for 10 minutes to separate serum and used for analysis of uric acid and Vitamin D.

Method

Determination of Serum Uric Acid

Uric acid is oxidized to allantoin by uricase with

production of hydrogen peroxide. The peroxide reacts with 4-amino antipyrine in presence of peroxidase to yield quinoneimine dye. The absorbance of this dye at 546 nm is proportional to uric acid concentration in the sample.

Estimation of Vitamin D by Chemiluminiscence Method

Sample antigen and purified 25-OH Vitamin D antigen competes to combine with 25-OH vitamin D monoclonal antibody to form antibody-antigen complex with starter reagent, the flash chemiluminiscent reaction is initiated. The light reaction is measured by a photomultiplier which is proportional to the concentration of vitamin D present in sample.

Statistical Analysis

Statistical software SYSTAT version-12 (by Cranes software, Bangalore) was used to analyze the data. The result were expressed in mean ± Standard Deviation (Mean± SD) Data was analysed by descriptive statistics as mean, SD, percentage etc. Comparisons of study group to control group by using the Students't' test. Pearson's correlation coefficient was used to find out the correlation between two variables. P – Values of <0.001 was considered as statistically significant.

Result

Table 1 showed that, the mean serum uric acid levels in essential hypertension was 6.98 ± 1.51 and in controls it was 4.72 ± 1.83. The mean serum uric acid in essential hypertension was significantly, higher when compared with healthy controls (p<0.001). As shown in Table 1 the mean serum Vitamin D levels in essential hypertension was 17.05 ± 7.13 and in controls it was 34.2 ± 5.18. The mean serum Vitamin D essential hypertension was significantly decreased in essential hypertension when compared with normal healthy controls (p<0.001).

Table 3 and 4 Showed thatcorrelations between the parameters. 'r ' values were for Uric acid Vitamin D verses hypertension. This illustrates that correlation between uric acid and diastolic and systolic blood pressure was positively correlated and significant. Correlation between Vitamin D and diastolic and systolic blood pressure was negatively correlated and non-significant.

Table 1: Baseline characteristic and biochemical changes in essential hypertension and control

Variable	Controls (n=45)	Essential hypertension (n=45)	P value
Age (In years)	27-72	25-74	-----
Sex (M / F)	30 / 18	29 / 27	------
Pulse rate	71.65±2.09	87.73±18.31	**<0.01**
Diastolic blood pressure	74.83±5.79	84.95±21.71	**<0.01**
Systolic blood pressure	112.25±8.54	132.48±27.25	**<0.01**
Uric acid (mg / dl)	4.72±1.83	6.98± 1.51	**<0.001**
Vitamin D	34.2±5.18	17.05±7.13	**<0.001**

Table 2: Pearson's correlation between the uric acid and hypertension

Parameters	Correlation Co- efficient	P-value
Systolic blood pressure	0.32	0.03 Significant
Diastolic blood pressure	0.32	0.03 Significant

Table 3: Pearson's correlation between the Vitamin D and hypertension

Parameters	Correlation Co- efficient	P-value
Systolic blood pressure	-0.2	0.16 Non-Significant
Diastolic blood pressure	-0.2	0.16 Non-Significant

Disccussion

Hypertension is an increasingly important medical and public health issue worldwide affecting approximately one billion individuals [10]. Because of risk factor for cardiovascular and renal morbidity and mortality, it is a leading contributor to global disease burden [11].

Uric acid has been implicated in hypertension through the probable role. It is thought to play in mediating hypertension via mechanisms like inflammation, vascular smooth muscle cell proliferation in renal microcirculation, endothelial dysfunction and activation of renin-angiotensin aldosterone system [8].

Vitamin D plays a key role in regulation of blood pressure and in the pathogenesis of hypertension

through its effects on calcium homeostasis, vascular smooth muscle, endothelial cells and activity of renin- angiotensin system [12]. Vitamin D deficiency is widely prevalent across all ages, races, geographical regions and socioeconomic strata. It plays an important role in skeletal development and calcium homeostastis [5].

In present study, the mean serum uric acid was significantly higher in essential hypertension when compared with normal healthy controls ($p<0.001$). Correlation between uric acid and diastolic and systolic blood pressure was positively significant. Our results are strongly supported to previous results. Charies et al have demonstrated that, hyperuricemia influenced the development of hypertension via its role in vascular endothelial cell dysfunction and activation of renin-angiotensin system [13]. Infollow-up Study, ≥40 years allowed to assess the durability of the prospective association of uric acid level with hypertension [14].

In current study, the mean serum Vitamin D was significantly decreased in essential hypertension when compared with normal healthy controls ($p<0.001$). Correlation between Vitamin D and diastolic and systolic blood pressure was negatively correlated and non-significant. Our results were similar to previous reports. Scragg et. al in their cross – sectional study showed that, significant inverse correlation with both systolic ($p<0.01$) and diastolic ($p<0.05$) blood pressure [15]. This association was stronger in patients who were more than 50 years. In one more cross- sectional study, Martin et.al reported that increased prevalence of hypertension in the lower quartile of 25 (OH) Vitamin D [16]. Thomas J. Wang et al have demonstrated that, potential interaction occurred between Vitamin D deficiency and hypertension. Left ventricular hypertension and vascular remodeling are major complication developed in hypertension. Thus Vitamin D deficiency directly promotes the development of hypertension [17].

Conclusion

Thus it can be concluded from the present study that, the essential hypertension is associated with abnormalities in the level of serum uric acid and Vitamin D. Because of association of Vitamin D deficiency and increased risk of hypertension its supplementation may play key role in controlling high blood pressure and to prevent further complication. The study also concluded that, serum uric acid and vitamin D can be used as biochemical markers to determine severity of hypertension and it may beneficial for better management and for developing new treatment strategies.

References

1. Kearney PM, Whelton M, Reynolds K, et.al. Global burden of hypertension analysis of worldwise data. Lancet. 2005; 365: 213-217.
2. Naomi DL Fisher, Gordon H. Williams. Hypertensive vascular diseases. Harrison's principles of internal medicine. 16th edvol 1: 1463
3. B. Wizner, B Gryglewska, J Gasowski et al. Normal blood pressure values as perceived by normotensive and hypertensive subjects. J Hum Hypertens. 2003; 17: 87-91.
4. Colin J, Dollery MB. Risk predictors, Risk indicators and benefit factors in hypertension. Am. J. Med. 1987; 82: 2-8.
5. T Reynolds. Serum uric acid, the endothelial and hypertension: an association revisited. J Hum Hypertension. 2007; 2: 591-593.
6. J Plange- Rhule, R Phillips, JWA Chempoung et al. Hypertension and renal failure in Kumasi Ghana. J Hum Hypertens. 1999; 13: 37-40.
7. Harris ED, Budd RC, Firestein GS, Genorese MC, Sergent JS et al. Kalley's textbook of Rheumatology. 2005; 7thedn USA; Elsevier Saunders.
8. Zhang W,Sun K, Yang Y, Zhang H. et.al. Plasma uric acid and hypertension in a chinese community, propective study and meta-analysis. Clin Chem. 2009; 55: 2026-2034.
9. Sandeep Chopra, Davis Cherian, JubbinJ Jacob. The thyroid hormone, parathyroid hormone and vitamin D associated hypertension. Indian Journal of Endocrinology and Metabolism. 2011; 15(8): 354-360.
10. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA et al. Seventh report of the joint national committee on prevention, detection, evaluation and treatment of high blood pressure. Hypertension. 2003; 42: 1206-1252.
11. Add J, Smeeth L, Leon DA. Hypertension in sub-Saharan Africa: a systematic review. Hypertension. 2007; 50: 1012-1018.
12. Lu wang. Vitamin and Hypertension. N A J Med. Sci. 2009; 2(4):115-149.
13. Charles BA, Shriner D. et al. A Genome- wide association study of serum uric acid in African Americans. BMC Medical Genome. 2011; 4: 17.
14. Perlsteinet. al. Uric acid and the development of hypertension. The Normative aging study hypertension. 2006; 48: 1031-1036.

15. Martin LA. Vitamin D and Metabolic syndrome. Nutri. Rev. 2006; 64(11): 479-786.
16. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity and blood pressure in the third national health and nutrition examination survey. American Journal of Hypertension. 2007; 20(7): 713-719.
17. Thomas J. Wang et. al. Vitamin D deficiency and risk of cardiovascular disease. Am. J. Med. Sci. 2009; 338(1): 40-44.

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1	Dermatology International	2	5000	4500	500	450
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4	Indian Journal of Anatomy	3	8000	7500	800	750
5	Indian Journal of Ancient Medicine and Yoga	4	7500	7000	750	700
6	Indian Journal of Anesthesia and Analgesia	3	7000	6500	700	650
7	Indian Journal of Biology	2	5000	3500	400	350
8	Indian Journal of Cancer Education and Research	2	8500	8000	850	800
9	Indian Journal of Communicable Diseases	2	8000	7500	800	750
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13	Indian Journal of Genetics and Molecular Research	2	6500	6000	650	600
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15	Indian Journal of Library and Information Science	3	9000	8500	900	850
16	Indian Journal of Maternal-Fetal & Neonatal Medicine	2	9000	8500	900	850
17	Indian Journal of Medical & Health Sciences	2	6500	6000	650	600
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19	Indian Journal of Pathology: Research and Practice	3	11500	11000	1150	1100
20	Indian Journal of Plant and Soil	2	5500	5000	550	500
21	Indian Journal of Preventive Medicine	2	6500	6000	650	600
22	Indian Journal of Research in Anthropology	2	12000	11500	1200	1150
23	International Journal of Food, Nutrition & Dietetics	3	5000	4500	500	450
24	International Journal of History	2	6500	6000	650	600
25	International Journal of Neurology and Neurosurgery	2	10000	9500	1000	950
26	International Journal of Political Science	2	5500	5000	550	500
27	International Journal of Practical Nursing	3	5000	4500	500	450
28	International Physiology	2	7000	6500	700	650
29	Journal of Animal Feed Science and Technology	2	4100	3600	410	360
30	Journal of Cardiovascular Medicine and Surgery	2	10000	8600	910	860
31	Journal of Forensic Chemistry and Toxicology	2	9000	8500	900	850
32	Journal of Microbiology and Related Research	2	8000	7500	800	750
33	Journal of Orthopaedic Education	2	5000	4500	500	450
34	Journal of Pharmaceutical and Medicinal Chemistry	2	16000	15500	1600	1550
36	Journal of Social Welfare and Management	3	7500	7000	750	700
37	Meat Science International	2	5000	4500	500	450
38	New Indian Journal of Surgery	3	7500	6600	710	660
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45	Indian Journal of Surgical Nursing	3	5000	4500	500	450
46	Indian Journal of Trauma & Emergency Pediatrics	3	9000	8500	900	850
47	International Journal of Pediatric Nursing	3	5000	4500	500	450
48	Journal of Community and Public Health Nurisng	2	5000	4500	500	450
49	Journal of Geriatric Nursing	2	5000	4500	500	450
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51	Journal of Nurse Midwifery and Maternal Health	3	5000	4500	500	450
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Original Article

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Evaluation of Diabetes Status in the Urban Population of Jaipur: A Community-Based Survey

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Abstract

Diabetes is a metabolic disorder rapidly rising all over the globe at an alarming rate. Since last 3 decades the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe. Diabetes mellitus is condition of glucose embolic disorders of carbohydrate metabolism. The Centre for Disease Control and prevention (CDC) estimated the prevalence of 7.9% in adults or about 16.7 million people in 2001. Due to the undiagnosed cases the number may raise above 22 million. The increased prevalence estimates that the diabetes will globally affect about 300 million peoples till 2025. The statistics shows that this disease is one of the main threatening diseases of 21st Centaury. The diabetes prevalence is associated with the age and approximately half of all cases occur in the age of 55 years.

The present study established the facts which already researched and written by the various scientists of the World. In addition to that the fact that BMI which is the determinant of the overweight and underweight in society, also have influenced by the type of oil consumption, television watch time and late sleeping time. This study suggests that a particular age group of 25-55 is more prone to the metabolic disease like diabetes and hypertension. The epidemiological survey also have outcome that the females of age group 25-45 having some % of underweight also. The underweight 25-45 age group have Hb% on lower side suggesting the occurrence of anemia.

Keywords: Body Mass Index; Diabetes; Blood Glucose; Epidemiology; Hb%; Hypertension.

Introduction

The diabetes is disease known to the mankind since 1500 before Christ (BC). The first described cases are believed to be of type 1 diabetes. Indian physicians detected around the same time the disease and classified it as madhumeha or honey urine noting that the urine would attract ants. The term "diabetes" or "to pass through" was first used in 250 BCE by the Greek Apollonius of Memphis. Type 1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 CE with type 1 associated with youth and type 2 with obesity. The term "mellitus" or "from honey" was added by Thomas Willis in the late 1600s to separate the condition from diabetes insipidus which is also associated with frequent urination.

Ancient Greek physician Aretaeus of Cappadocia provided the first complete clinical description of diabetes and described that the excessive amount of urine which passed through the kidneys."(Dallas,

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John,2011)

Aretaeus did attempt to treat diabetes but could not give a good prognosis; he commented that "life (with diabetes) is short, disgusting and painful." (Medvei, Victor Cornelius,1993)

In medieval Persia, Avicenna (980–1037) given a detailed account on diabetes mellitus in The Canon of Medicine, "describing the abnormal appetite and the collapse of sexual functions," and he documented the sweet taste of diabetic urine. In addition to it, he also described diabetic gangrene, and treated diabetes using a mixture of lupine, trigonella (fenugreek), and zedoary seed, which produces a considerable reduction in the excretion of sugar, a treatment which is still prescribed in modern times. Avicenna also described diabetes insipidus very precisely for the first time, though it was much later that Thomas Willis differentiated it from diabetes mellitus in a chapter of his book Pharmaceutice rationalis (1674).

The World Health organization describes the Diabetes as a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

The Common Facts about Diabetes Includes

- More than 220 million people worldwide have diabetes.
- In 2005, an estimated 1.1 million people died from diabetes.
- Almost 80% of diabetes deaths occur in low- and middle-income countries.
- Almost half of diabetes deaths occur in people under the age of 70 years; 55% of diabetes deaths are in women.
- WHO projects the diabetes deaths will double between 2005 and 2030.
- Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of diabetes (W.H.O,2009)

The Common Consequences of Diabetes Includes

Over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves.

- Diabetes increases the risk of *heart disease and stroke*. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke).
- Combined with reduced blood flow, neuropathy in the feet increases the chance of *foot ulcers* and eventual *limb amputation*.
- *Diabetic retinopathy* is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind, and about 10% develop severe visual impairment.
- Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of *kidney failure*.
- *Diabetic neuropathy* is damage to the nerves as a result of diabetes, and affects up to 50% of people with diabetes. Although many different problems can occur as a result of diabetic neuropathy, common symptoms are tingling, pain, numbness, or weakness in the feet and hands.
- The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes.

The diabetes is becoming prevalent in society. India in last many decades became diabetes hub. The diabetes mellitus is developing in society due to metabolic misbalance and due to genetic factors. The life style is so changed over the time that it harnessed the stress, disturbances in eating and sleeping habits is responsible for the development of metabolism misbalance and result in the genesis of diabetes mellitus and hypertension. The present study has the aim of Evaluation of diabetes status in the urban population of Jaipur. The aim can be achieved by the evaluation study.

Material and Method

Study Design

There are two main types of the epidemiological studies i.e observational study and experimental study. In observational studies, the researcher observes and systematically collects information, but does not try to change the people (or animals, or reagents) being observed. In an experimental studies, by contrast, the researcher intervenes to change something (e.g., gives some patients a drug) and then observes what happens. In an observational study there is *no* intervention.

The basis of the present study is observational.

The data analysis collected through survey were analyzed using Stastical software and the blood samples collected were analyzed by available method using quality control. The study so designed that the survey, blood pressure measurement, data analysis are performed for genesis of results and documenting it.

Cross-Sectional Surveys

These are type of observational study. For example, To know the prevalence of diabetes in this community? A random sample of people and record information about their health in a systematic manner can be obtained. Compare people with, and without, diabetes in terms of characteristics (such as being overweight) that may be associated with the disease.

Cohort, or "Longitudinal", or "Prospective" Studies

These are like surveys, but extend over time. This allows to study changes and to establish the time-sequence in which things occur. Therefore, one can use this to study causes. For example, one could draw a sample of people (normal healthy people), and collect information on the factor one have hypothesized to be a cause of the disease.

Population under study An official Census 2011 provided the details population of Jaipur, a district of Rajasthan. In 2011, Jaipur had population of 6,626,178 of which male and female were 3,468,507 and 3,157,671 respectively. In 2001 census, Jaipur had a population of 5,251,071 of which males were 2,768,203 and remaining 2,482,868 were females. Jaipur District population constituted 9.67 percent of total Maharashtra population. In 2001 census, this figure for Jaipur District was at 9.29 percent of Maharashtra population.

There was change of 26.19 percent in the population compared to population as per 2001. In the previous census of India 2001, Jaipur District recorded increase of 32.40 percent to its population compared to 1991. (http://www.census2011.co.in/).

Rural Urban Jaipur

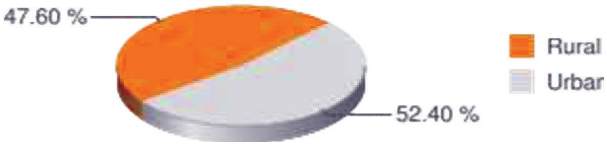


Fig. 1: Rural and Urban population in Jaipur District Ref:- http://www.census2011.co.in

Methods Used on Samples Collected

The materials used in this study includes the question sets, syringes, kits for blood glucose tests, The glucose test principle is based on Glucose Oxidase abd Peroxidase method, the Hemoglobin estimation is based on Cyanmeth Hemoglobin estimation method, autoanalayzer, lap top for onsite record of results and various related materials.

The method used in the present study is based on the questionnaire prepared for the documentation, measurement of Height, Weight, Blood Pressure, Fasting and Post-meal Blood Sugar Level, Hemoglobin % level.

The data then analyzed by applying statistics principles using MS-Excel for comparative analysis among groups of population divided on basis of age.

The Body Mass Index (BMI) was calculated. The biochemical, Medical data were correlated with the diet habits, stress level, diet timings.

The Doctor / Medical Practitioner recorded the medical history and other measurements like Height, Weight, Blood Pressure and took sample for blood analysis.

Results

Table 1: Showing the Mean and SD of the Blood Sugar level, Blood pressure and Hemoglobin in different age groups

Age Group	No of peoples	Blood Sugar(F) Mean±SD	Blood Sugar(PM) Mean±SD	Blood Pressure(Sys) Mean±SD	Blood Pressure(Dis) Mean±SD	Hemoglobin(%) Mean±SD
5-25	14	104.64±25.21	139±38	119.21±9.5	83.07±9.3	13.71±1.88
26-45	48	108.53±33.76	136.91±36.02	127.08±15.24	86.16±15.24	12.94±2.4
46-55	14	146.14±58.07	205±92.44	146.07±18.11	97.28±10.79	14.26±2.01
56-75	36	121.63±36.59	161.27±41.24	141.34±15	98.47±15.21	13.29±1.54
76-85	08	120.29±18.25	194±19.72	148.12±17	101.75±9.75	14.18±1.4
Total				**120**		

Table 2: Showing the Mean and SD of the Height, Weight and Body Mass Index (BMI) in different age groups

Age Group	No of peoples	Height(in meter) Mean±SD	Weight(in Kgs) Mean±SD	BMI Mean±SD
5-25	14	1.52±0.52	48.64±11.62	20.71±2.8
26-45	48	1.59±0.065	86.16±15.24	22.95±4.34
46-55	14	1.62±0.039	68.07±11.74	25.71±4.24
56-75	36	1.60±0.26	64.22±9.06	24.90±3.15
76-85	08	1.63±3.72	61.12±11.77	22.90±3.5
Total		**120**		

Table 3: Showing the numbers of Males, females, and diet habits

Age Group	No of peoples	Males	Females	Veg. 1	Non Veg. 2	Veg+Eggs 3
5-25	14	06	08	09	03	02
26-45	48	14	34	31	06	11
46-55	14	08	06	12	----	02
56-75	36	19	17	28	----	08
76-85	08	07	01	05	----	03
Total	**120**	**54**	**66**	**85**	**09**	**26**

Table 4: Showing BMI of the people as per age group

Age Group In years	No of peoples	% of peoples having BMI Below 18	% of peoples having BMI 18-22	% of peoples having BMI 22.1-27	% of peoples having BMI 27.1-32
5-25	14	-----	78.57	21.42	-----
26-45	48	12.51	16.63	35.41	35.41
46-55	14	---	21.42	57.16	21.42
56-75	36	---	16.67	61.11	22.21
76-85	08	---	37.5	50	12.5

Body Mass Index (BMI)

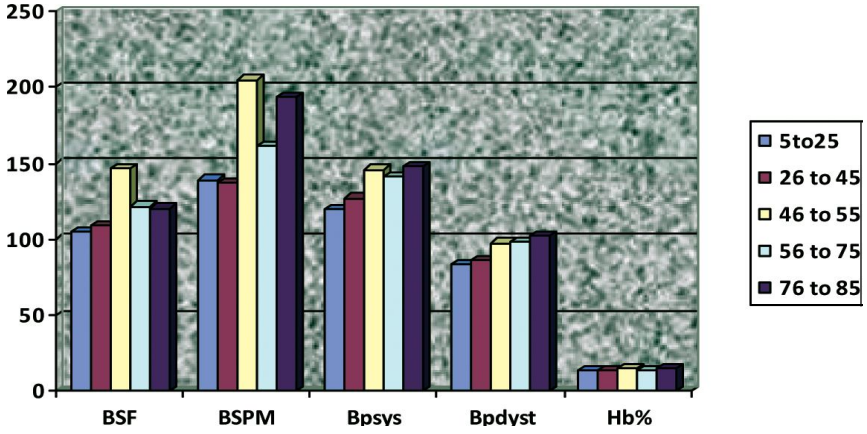
BMI is based on your height and weight. Underweight: BMI is less than 18.5, Healthy weight: BMI is 18.5 to 24.9 Overweight: BMI is 25 to 29.9(http://www.webmd.com/men/weight-loss-bmi,accessed on 23-04-2016)

Table 5: Showing Average BMI, Television watch time, sleeping time and cooking oil type used

Age Group	No of peoples	Average BMI	% of Underweight And Overweight	Television Watch time No and % of More than 3 hr daily viewer	Sleeping time No and % sleeping at 11pm or later	Cooking Oil Consumption Soybeans(Sy) Musturd(M) Sunflower(Sf) Rice bran (R)
5-25	14	20.71	7.14% Overweight	00	10(71.42%)	Sy 10 M 02 Sf 02 R 00
26-45	48	22.95	Underweight 14.58% Overweight 27.0%	6(12.5%)	27(56.25%)	Sy 27 M 12 Sf 09 R 00
46-55	14	25.71	Overweight 57.14%	03(21.42%)	07(50%)	Sy 4 M 4 Sf 5 R 1
56-75	36	24.90	Overweight 41.66%	8 (22.22%)	07(19.44%)	Sy 25 M 04 Sf 07 R 00
76-85	08	22.90	Overweight 7.14%	00	00	Sy 4 M 1 Sf 3 R 00

The results tabulated shows that the population of age group 46-55, 56-75 and 75+ under study is more vulnerable to the diabetes and hypertension. The high values in the Standard Deviation suggestive of the wide range of the readings. It also indicates that some people under the study have severe diabetes and hypertension. The body mass index is suggestive of the age group 46-55 is on border of overweight while the age group 5-25 is underweight. In the age group 5-25, 78.57% people underweight while in age group of 26-45, 46-55, 56-75, 75-85 the 35.41%, 21.42%, 22.21% and 12.5% people were found obese. This obesity is associated with increased hypertension, increased blood sugar level and associated disorders. The various observations have also correlated with the daily routine, food habits also.

The results in Table 5 are suggestive of the increase in Body Mass Index is associated with the television watching time, sleeping time on regular basis and type of oil consumed. The more the person is seated while watching television at home more obesity will be observed. The type of oil is also play important role. Soybean oil and mustard oil was consumed by large population in the cases of high overweight age groups.



BSF—Blood Sugar fasting, BSPM— Blood Sugar Post Meal, Bpsys— Blood Pressure Systolic, Bpdyst— Blood Pressure Diastolic, Hb%— Hemoglobin %

Fig. 2: showing age groups (Y axis) and Blood sugar-Fasting and post meal, Blood Pressure-Systolic and Diastolic and Hb%(X axis).

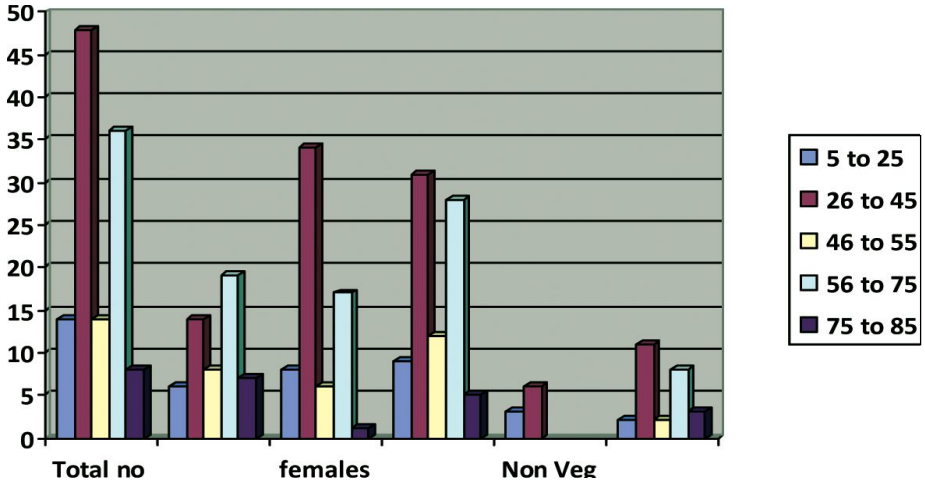


Fig. 3: Showing age groups (Y axis) and total numbers of people, males , females , veg, non-veg and veg+eggs (X axis).

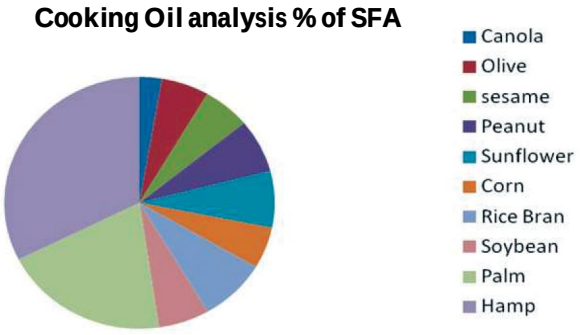


Fig. 4: Showing Saturated fatty acid % in various cooking oils

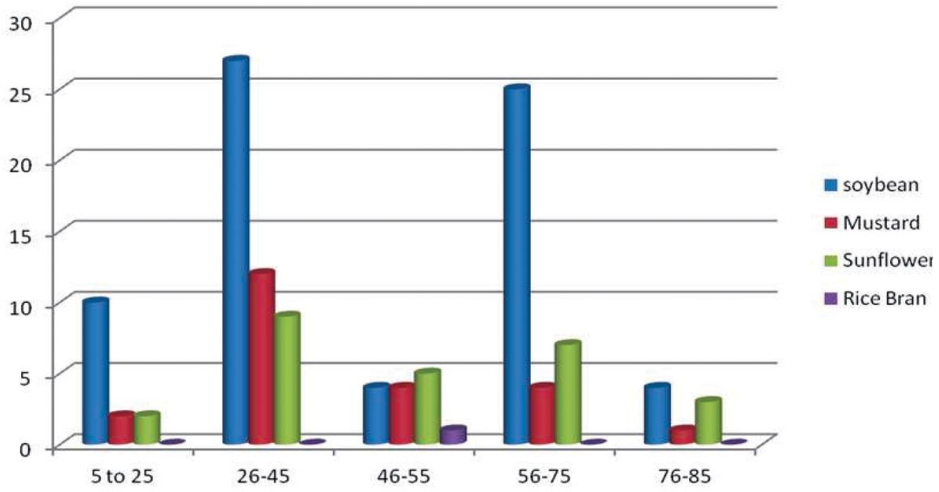


Fig. 5: Numbers of people taking different oils-analysis chart

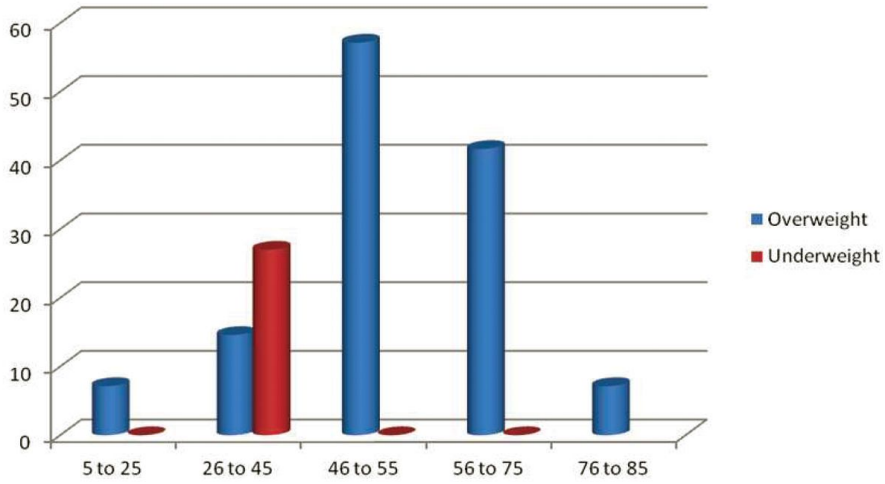


Fig. 6: Showing the Overweight and Underweight % among population under study

Discussion

In India the disease prevalence is growing fast so now India becoming a capital of diabetes. This situation is arising due to lack of active life and changes in life style of public. The causes of the development of diabetes are due to sedentary work, lack of exercise, unbalanced diet intake and hereditary transfer. The various factors also play direct or indirect roles in the development of diabetes includes alcohol intake, stress level, diet habits, work, regular exercise, hereditary history, smoking and so on.

In the present study demographic profile of

community will be studied based on questionnaire survey. The questions were well designed to obtain the information for assessment of the prevalence of diabetes in the population. The prevalence of diabetes will be further classified depends on age, sex, marital status, literacy level, occupation, diet habits-like vegetarian, non vegetarian, vegetarian with eggs, food habits inclusion of milk in food, grains, meat type used, sanitation habits, health status based on questions, family history, physical examinations-height, weight, blood pressure, etiology of disease occurrence, laboratory investigations-hemoglobin, blood sugar-fasting and post meal.

The objective of the study includes assessment of prevalence of diabetes in population under study. Hemoglobin level, Body Mass Index and correlation between food habits, occupation and diabetes status in community were performed in the present study.

These data was collected for the sample size of 120 and statically evaluated for significance as well as for understanding the problem severity in the population under study.

India diabetes is coming up as an epidemic. In order to understand the true extent of the problem and its impact on diabetes care, there is a need to review the epidemiology of diabetes from different regions of India. The epidemiology of diabetes in India has an extensive history. The earliest national study reported an overall prevalence of 2.1 % in urban areas and 1.5% in rural areas. (V. mohan and R. pradeepa, 2009)

The study conducted by Gupta R *et al*, concluded that the in last two decades, there has been a marked increase in the prevalence of diabetes among both urban as well as the rural Indians. There is sharpest increase seen in the Southern India. Subsequent studies confirmed this high prevalence of diabetes in urban south India. Although in rural India the prevalence of diabetes is much lower than in the urban population, even here the prevalence rates are rapidly rising, though clearly more studies are needed. Variations in the prevalence rates of diabetes in different urban populations of India are expected because of the large variation in the prevalence of cardiovascular risk factors in different regions and states (Gupta R, 2005, Gupta R *et al*, 2006).

The study conducted by the Bandana Sachdev in 2011 concluded that the prevalence of pre-diabetes and diabetes in the tribal population was found to be higher than that in non-tribal population in Rajasthan. The relative independent contribution of excess adiposity, as indexed by measures of weight and square of height i.e. BMI known to be an modifier risk factor for obesity related ill health. Advancing age and liquor consumption might play associated role in the development of Type 2 diabetes mellitus and hypertension. The prevalence rate of diabetes and its complications is increasing continuously among these communities due to lack of access to diabetes care and knowledge.

The question answer based survey study is very popular in social science. It gives a rough picture of the event happening among the society. The accuracy and correctness of the answers always remains under the doubt but may be correlated with the experimental facts. In the present study the questions asked and the answers replied correlates with the facts observed in the measurements. Those age groups who have hampered life style, more stress level have diabetes and hypertension. The severity of the disease is seen more in the age group of working age and hence indicating that the population at working age. As the maximum peoples examined were vegetarian hence it shows that the food type is not so importance rather diet time, stress level, diet consumption, exercise in daily routine are the main factor which plays role in development of diabetes.

The study is unique in its way as in this study it is tried to correlate the diet timings, television watch timings, use of cooking oils type, ghee type with the onset of diabetes among the society.

In his review article, S Ramnathan Iyer in 2002, he wrote about the sleeping time and clinical implications on Type 2 diabetes. Sleep is essential for life. Body systems require sleep of good quantity and quality for their proper functioning. Glucose metabolism can be affected adversely by many sleep disorders. Obstructive sleep apnea (OSA) is one of the most important disorders identified in the last 50 years which has systemic effects including glucose metabolism. Aging process also has its effects on glucose metabolism. There is a close relation between sleep, aging and metabolic syndrome. OSA and Type 2 Diabetes Mellitus (Type 2 DM) share several underlying factors in common. There are facts to show a close association between sleep deprivations, sleep disordered breathing-OSA, excessive sleepiness, insomnia, restless legs syndrome and Type 2 DM. The role of sleep deprivation, in the genesis of obesity needs to be recognized. The close association of OSA with insulin resistance demands the recognition of OSA in fatty liver and polycystic ovary syndrome. Treatment of OSA by continuous positive airway pressure has been shown to increase insulin sensitivity. It is important for primary care physicians to have a high degree of suspicion of an underlying sleep disorder in patients with diabetes.

Management of sleep disorder is highly rewarding.

The reduced sleep times on regular basis are associated with obesity (Haster G *et al.*2004) Sleep deprivation induced stress has a role to play in the development of obesity. Sleep deprived persons have daytime sleepiness and have a tendency to overeat and eat fast. Intake of food in various forms, helps the sleep deprived persons to overcome daytime sleepiness. Chewing tobacco, smoking also drive away sleep but are risk factors for type 2 diabetes (Iyer SR,2000). Chronic sleep restriction coupled with eating contributes separately to the development of obesity. It is not uncommon to find nap pod in commercial organizations where employees can take a power nap to boost their performances. (S Ramnathan Iyer, 2004)

The present study also suggests same by question answer analysis. The persons who sleep late in night have overweight and tend to develop hypertension and diabetes.

Healthy Cooking Oils

Cooking oils are made of three types of fat. Saturated fatty acids (SFA) cause oxidative stress: a process which leads to cancer cell damage and destruction, as well as being fundamental in the aging process. Monounsaturated fatty acids (MUFA), on the other hand, are the good fatty acids.

The American Heart Association say that our energy intake should consist of 10% SFA 15% MUFA and 10% (Polyunsaturated fatty Acids) PUFA. But ideally one should try to reduce our fat dependency to < 30%.

Body Mass Index (BMI)

BMI is a simple calculation using a person's height and weight. The formula is BMI = kg / m^2 where kg is a person's weight in kilograms and m^2 is their height in meters squared. A BMI of 25.0 or more is overweight while the healthy range is 18.5 to 24.9. BMI applies to most adults 18-65 years. BMI is not used for muscle builders, long distance athletes, pregnant women, the elderly or young children. This is because BMI does not take into account whether the weight is carried as muscle or fat, just the number. (www.diabetes.ca accessed on 01-05-2016)

BMI in present study compared with many parameters like television watch time, type of oil consumed, and sleeping time. The observation shows that the sybean oil consumer, late night sleeping persons and more than 3 hours television viewers have increased BMI. All these activities together increase the weight of a person and thus increasing the BMI.

Age also plays important role as the increase in BMI is observed in the age group of 26-45 and 46-55. An underweight % of the 14.58% in the age group of the 26-45 indicates that the women have lesser BMI also, as in this age group out of 48 persons 34 were females. This is mixed picture of the group where some females were suffering from underweight and anemic situation too.

Type 2 diabetes is being observed in the young popula-tion of developing countries, which causes a large burden on individuals and the society. Therefore, prevention of diabetes should be considered as a priority as follows:

Development and evaluation of healthy lifestyle plans, focusing on the following aspects: Prevention and early treatment of overweight and obe-sity, especially in high risk groups. Consume a nutritious diet including low-fat content, especially saturated fat, no sugar and high nutritional supplementary proteins.

Follow active lifestyle including regular physical activity at least an hour a day, and vigorous activities necessary to reduce the risk of type 2 diabetes.

Summary and Conclusion

The study established the facts which already researched and written by the various scientists of the World. In addition to that the fact that BMI which is the determinant of the overweight and underweight in society, also have influenced by the type of oil consumption, television watch time and late sleeping time. This study suggests that a particular age group of 25-55 is more prone to the metabolic disease like diabetes and hypertension. The epidemiological survey also have outcome that the females of age group 25-45 having some % of underweight also. The underweight 25-45 age group have Hb% on lower side suggesting the occurrence of anemia. The more intense research with large population is the need for more concrete conclusion. The age group of 46-55 and 56-75 affects by the hypertension. The severity prevails with the age as per the results shown. The development of the disease diabetes and hypertension took place in early 25-45 years as in that age large numbers of the people generally avoid regular exercise and concentrate more on work. So impaired metabolism due to variation of time in eating, sleeping and stress tend to develop diabetes and hypertension. The regular exercise in form of Yoga and proper intake of water,

reduction in stress level, timely eating and sleeping habits are some actions need to be incorporate into the life style for 20,30,40 years of life so that in old age the metabolic disorders will be avoided.

Recommendations

The Diabetes mellitus develop when a person follow irregular life style in terms of sleeping, eating time, eating habits of junk foods, stress level, genetic factor, lack of daily exercise. The disease is connected with the hypertension and obesity. As it is well said that the "precaution is better than cure". Hence, there are some precautionary measures to prevent onset of Diabetes mellitus.

1. Always eat, sleep on time.
2. Avoid stress in daily life.
3. Do exercise daily.
4. Eat salad and dietary fibers more.
5. Choose best oil for cooking food.
6. Stop watching television 2 hours before going to bed.
7. After attaining 40 years of age yearly do medical checkup in good Hospital.
8. Avoid eating junk foods and fast foods.
9. Be active in work and in home.
10. Take food supplements, if required.

References

1. Ahuja MMS. Epidemiological studies on diabetes mellitus in India. In: Ahuja MMS (ed) Epidemiology of diabetes in developing countries. Interprint, New Delhi. 1979; 29-38.
2. Bandana Suchdev. Community Based Study on Incidence of Type 2 Diabetes and Hypertension among nomad tribal population of Rajasthan, India, I.J.S.N., 2011; 2(2): 296 - 301. ISSN 2229 – 6441
3. Chakravarthy A. A study of diabetes and its treatment with special reference to Bengalees and their diet. Indan Med Rec. 1938; 58: 65.
4. Dallas, John. "Royal College of Physicians of Edinburgh. Diabetes, Doctors and Dogs: An exhibition on Diabetes and Endocrinology by the College Library for the 43rd St. Andrew's Day Festival Symposium". 2011.
5. Haster G, Busysee DJ, Klaghofer R etal. The association between short sleep duration and obesity in young adults : 13 year prospective study sleep. 2004; 27: 661–6.
6. http: / / mensurations.com / healthy-cooking-oils / accessed on 01-05-2016
7. http: / / www.census2011.co.in / census / district / 435-jaipur.html / accessed on 07-07-2016
8. Iyer SR. Type 2 Diabetes Express Highway, where is the 'U' turn? J Assoc Physicians India. 2003; 51: 495-500.
9. Medvei, Victor Cornelius. The history of clinical endocrinology. Carnforth, Lancs., U.K: Parthenon Pub. Group. 1993; pp. 23–34. ISBN 1-85070-427-9
10. Ranjita Mishra. Editor (2011), Indian Foods: AAPI's Guide To Nutrition, Health and Diabetes (Second Edition).
11. S Ramnathan Iyer. Sleep and Type 2 Diabetes Mellitus- Clinical Implications, JAPI. OCTOBER 2012 Oct; 60: 42-47
12. Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance. In: Gan D, editor. Diabetes Atlas. International Diabetes Federation. 3rd ed. Belgium: International Diabetes Federation. 2006; 15-103.
13. V. Mohan, S. Sandeep, R. Deepa, B. Shah & C. Varghese. Epidemiology of type 2 diabetes: Indian scenario, diabetes Indian J Med Res. 125, March 2007; 125: 217-230.
14. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27: 1047-53.
15. www.diabetes.ca accessed on 01-05-2016)
16. World Health Organization Report, 2009

Revised Rates for 2016 (Institutional)

Title	Frequency	Rate (Rs): India	Rate ($):ROW
Dermatology International	2	5000	500
Gastroenterology International	2	5500	550
Indian Journal of Agriculture Business	2	5000	500
Indian Journal of Anatomy	3	8000	800
Indian Journal of Ancient Medicine and Yoga	4	7500	750
Indian Journal of Anesthesia and Analgesia	3	7000	700
Indian Journal of Anthropology	2	12000	1200
Indian Journal of Biology	2	4000	400
Indian Journal of Cancer Education and Research	2	8500	850
Indian Journal of Communicable Diseases	2	8000	800
Indian Journal of Dental Education	4	4500	450
Indian Journal of Forensic Medicine and Pathology	4	15500	1550
Indian Journal of Forensic Odontology	2	4500	450
Indian Journal of Genetics and Molecular Research	2	6500	650
Indian Journal of Law and Human Behavior	2	5500	550
Indian Journal of Library and Information Science	3	9000	900
Indian Journal of Maternal-Fetal & Neonatal Medicine	2	9000	900
Indian Journal of Medical & Health Sciences	2	6500	650
Indian Journal of Obstetrics and Gynecology	3	9000	900
Indian Journal of Pathology: Research and Practice	3	11500	1150
Indian Journal of Plant and Soil	2	5500	550
Indian Journal of Preventive Medicine	2	6500	650
International Journal of Food, Nutrition & Dietetics	3	5000	500
International Journal of History	2	6500	650
International Journal of Neurology and Neurosurgery	2	10000	1000
International Journal of Political Science	2	5500	550
International Journal of Practical Nursing	3	5000	500
International Physiology	2	7000	700
Journal of Animal Feed Science and Technology	2	4100	410
Journal of Cardiovascular Medicine and Surgery	2	9100	910
Journal of Forensic Chemistry and Toxicology	2	9000	900
Journal of Microbiology and Related Research	2	8000	800
Journal of Orthopaedic Education	2	5000	500
Journal of Pharmaceutical and Medicinal Chemistry	2	16000	1600
Journal of Practical Biochemistry and Biophysics	2	5500	550
Journal of Social Welfare and Management	3	7500	750
New Indian Journal of Surgery	3	7100	710
Ophthalmology and Allied Sciences	2	5500	550
Otolaryngology International	2	5000	500
Pediatric Education and Research	3	7000	700
Physiotherapy and Occupational Therapy Journal	4	8500	850
Urology, Nephrology and Andrology International	2	7000	700
SUPER SPECIALITY JOURNALS			
Indian Journal of Emergency Medicine	2	12000	1200
Indian Journal of Surgical Nursing	3	5000	500
Indian Journal of Trauma & Emergency Pediatrics	3	9000	900
International Journal of Pediatric Nursing	3	5000	500
Journal of Community and Public Health Nurisng	2	5000	500
Journal of Geriatric Nursing	2	5000	500
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Review Article

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Indispensable Role of Protein in Cancer

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Abstract

Proteins are one of the most dynamic and versatile biomolecules which present in all the organisms. Protein plays a pivotal role in cell growth, cell motility, and biosignalling, provide immunity as well as cell recognition and many more biological processes like replication, transcription and repair. Transcription factors are also protein which present in vicinity of various DNA sequences and by binding to these sequences they can upregulate or downregulate the expression of the particular gene. Some protein performs more than one function in body and some are specific for a particular function in the biological system. Cancer is one of the deadly diseases of present century. Cancer is abnormal growth of cells which can be generated by multiple unlimited cell divisions and form a lump or tumor which can be benign or malignant. At present era the role of protein in cancer is very much skeptical that weather it enhance metastasis or suppress the effect of cancer. Cancer is the second most deadly disease of century after the heart disease. This mini review is an effort to give a glimpse of indispensable role of protein in cancer.

Keywords: Protein; Cancer; F-Box Protein; Cell Proliferation; Apoptosis; Metastasis.

Introduction

Protein is a very versatile macromolecule which is essential to the entire organism. Protein plays crucial role in growth, regeneration, differentiation of cell, cell signaling, transmission of nerve impulse, in cell cycle, storage and various biological processes [1]. Besides these function proteins can also participate in various biological processes like replication, transcription, recombination and repair. Various transcription factors present in promoter and other genomic locations of a gene recognize specific DNA sequences and after binding to these they upregulate or downregulate a gene expression [2,3]. When normal gene expression of cells takes place it might contribute to the regular growth and normal functioning of the cell whereas its overexpression or down expression or expression of a defected protein, in neoplastic cells may cause abnormal tumor growth [4]. Many protein-encoding genes which regulate cell division and differentiation may undergo mutation and results into the abnormal behavior of neoplastic cells. With course of time more genes undergo mutation because the genes which produce proteins that usually repair DNA damage are themselves not functioning normally due to the mutation. Successively mutations begin to spread in the cell which leads to abnormalities in cell and daughter cells. Out of the whole genome only a small number of genes have been categorized under cancer causing genes. These malfunctioning genes can be classified as proto-oncogenes (which generate protein product for enhancing cell division and inhibit apoptosis),

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oncogenes (the genes which undergo mutation) and tumor suppressor (which synthesize proteins inhibit cell division and cause apoptosis) [5]. In normal cells controlled cell growth is regulated by maintenance of proto-oncogenes as well as tumor suppressor because proto-oncogenes enhance cell growth while later one slow down the cell growth. In normal cells, nucleus receives a signal for stimulation of cell division by a protein that is encoded by proto-oncogenes. These proteins are known as signaling proteins [6]. There are various proteins which are involved in signaling process and many other biological events. The substantial roles proteins play in other biological events are discussed in further section.

Role of Protein in Augmentation and Suppression of Cancer

Proteins are the main artist of the theater which is cell of the organism. It carries out various biological processes by interacting with other molecules such as DNA, RNA, small molecules, drugs as well as other proteins via binding sites present as binding pocket in proteins. Earlier it was discussed that protein take part in cell signaling and various other proteins play substantial role in signal transduction pathways. Cell growth is regulated by various cell signal transduction pathways, so their inhibition might be a factor of tumor pathogenesis. Activity of protein in signaling pathways can be hampered by phosphorylation which is achieved by the action of protein kinase [7]. Imanitib was reported as first anticancer agents targeted on a protein kinase for inhibition of the oncogenic kinase BCR-Abl and it is also participated actively in chronic myelogenous leukemia [8]. Protein Kinase A (PKA) is belongs to the serine-threonine protein kinase superfamily, which is recognize as cAMP-dependent protein kinase which perform signal transduction by binding to the cAMP. cAMP is found in almost all cells and it is produced from ATP by adenylate cyclases. It was used by a number of harmones, signal substances and neurotransmitters for sending messeage to intracelleular environment and this is the reason that rate of cAMP production is dependent on extracellular as well as intracellular signals. In cell it might play a pivotal role in activity of different proteins. In eukaryotes cAMP play a major role in activation of PKA.

Stork et al [9] and Insel et al [10] has been reported role of cAMP/PKA pathway in stimulation of cell growth in many cells as well as inhibiting in some other cells. Regulation of cell proliferation was intimidated by involvement of PKA action on transcription factors [11]. It was established by various group that dysregulation of PKA signaling might cause various types of cancer such as lung cancer, endocrine tumors as well as prostate cancer [12]. So, it was suggested that abnormal PKA should be investigated for diagnosis and treatment of cancer in patients. Lin et al has reported that another protein Ki67 by interacting with other nuclear protein NIFK play an important role in tumor formation in various organs such as breast, lung, brain and prostate gland [13]. But the exact mechanism by which cancer cells proliferate by involvement of this protein has become a riddle for scientists. In another report PIWI protein which is a subfamily of Argonaute protein family was proposed to be involved in tumor formation and its proliferation in breast. PIWI proteins are specifically play role in stem cell regeneration and germline development in various organisms [14].

Chen et al has reported earlier that S100 gene family protein is also play a substantial role in cancer formation and its progression. This family is specifically involved in calcium binding, calcium homeostasis, cell growth and migration, cell cycle and regulation of transcription factors [15]. By involvement of various biological functions this protein has a close relation with metastasis and cancer proliferation. Cells use its cytoskeleton for cell motility, polarization and division and the most important cytoskeleton protein is known as Actin-filament-bundling protein. The actin cytoskeleton represents an important mess of proteins that encroach on invasion, motility, polarity, survival and growth of normal cells, and as such is often sabotaged by cancer cells. Abnormal cancer cells use this actin protein to invade through the surrounding tissue and travel in lymphatic and vascular system and via this spread cancer in other tissues and organs [16]. Various proteins which participated in cancer formation or suppression is demonstrated by (Figure 1).

Inspite of protein which involve progression of cancer some proteins are found to be tumor suppressing. Insulin-like growth factors (IGFs) are a group of peptides which are involved in various cell processes like cell differentiation, proliferation and apoptosis; make it a strong contestant for the tumorigenesis. Two important peptides of this family are IGF-I and IGFBP-3, both are specifically dependent on growth hormone but also influenced by age, nutrition and sex of the organism. It was established by various group that high circulating concentration of IGF-I is associated with enhancement of cancer formation whereas IGFBP-3 showed contrasting results. Its high concentration involved in the reduced chance of cancer [17, 18].

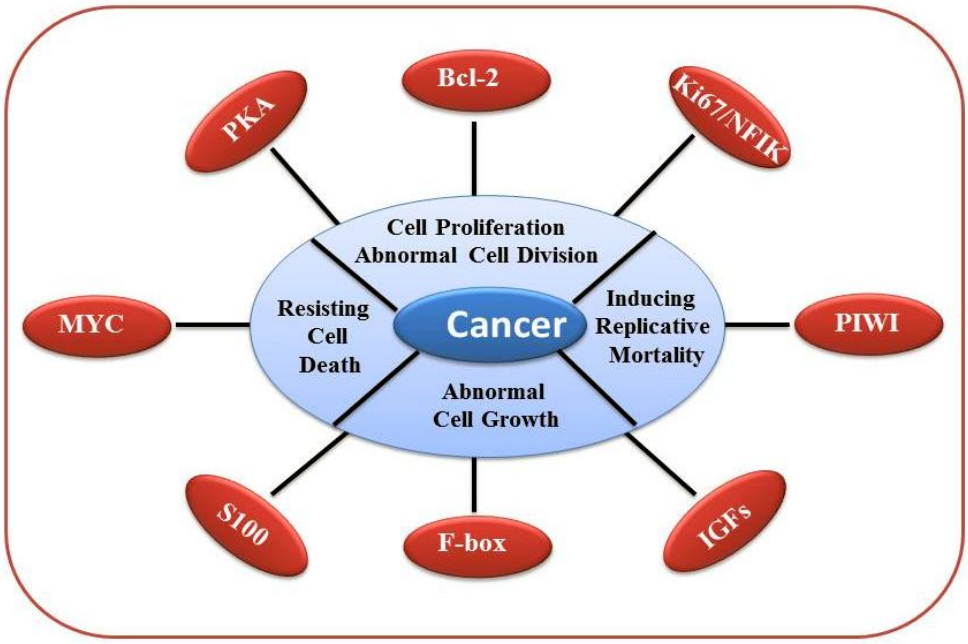

Fig. 1: Schematic representation of various proteins involve in cancer proliferation and suppression

IGF-I is considered to be antiapoptotic and mitogenic while IGFBP-3 as tumor growth inhibitor. Renhen et al has suggested that nutrition and life style also affects the circulation and function of these peptide growth hormones [19]. Wang et al has reported that human malignancies are expanded by proteolysis intervened by F-box proteins. These proteins play a pivotal role in cell cycle regulation. FBXW7 one of the F-box protein has already well-studied tumor suppressor that act and degrade various oncogenic proteins [20]. Sreedhar et al has also established in his report that heat shock proteins (Hsps) play as significant pharmacological target to encounter cancer. The important function played by Hsps in immune signaling and its innate quality make them potential target for tumor suppressant study [21]. There are many more proteins such as Bcl-2 family [22], LARP family protein [23], MYC family of protein [24], NEET protein [25] and Dynamin-related Protein 1 (Drp 1) [26] all are related with one or more than one type of cancer. These proteins plays substantial role in cancer proliferation as well as cancer progression of various tissues and organs such as lung, breast etc. Specifically Bcl-2 and MYC are the major class of proteins which regulate the cell proliferation and apoptosis.

Conclusion

The role of protein is indispensable in a number of biological processes such as transcription, cell differentiation, cell growth, storage, cell division and cell signaling. Various proteins play a pivotal role in tumor progression and proliferation whereas some protein acts as dysregulation of cancer and tumor suppressant. Cancer has become the most deadly and serious disease at present era due to instant mutation in gene and abnormal growth of cells. It is need of the hour to identify the proteins which involve directly or indirectly in the cancer formation. Other factors like environment, life-style, tobacco and some steroids also play major role in causing cancer and metastasis. Individual with history of cancer in family should be tested for cancer on regular basis. Early diagnosis can increase the survival rate of patient and as we all are aware about prevention is better than cure we should change our life-style and say no to all the steroids and tobacco. This review is a small effort to shed light on role of protein on the cancer proliferation and progression which might give an insight to investigate other proteins for regulation and deregulation of genes and via targeting and alteration of these proteins cancer formations can be hampered.

Conflict of Interest

Author is confirming that there is no conflict of Interest in this article.

References

1. Nelson DL, Cox MM. Lehninger's Principles of Biochemistry (4th ed.) New York, New York: W. H.

Freeman and Company 2005.

2. Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP et al. Molecular Cell Biology (5th ed.). New York, New York: WH Freeman and Company 2004.
3. Dickerson, RE, Geis I. The structure and action of proteins. Benjamin/Cummings, Menlo Park, California 1969.
4. Bahls C, and Fogarty M. An outline of several approaches to controlling cancer. The Scientist. 2002; 16(11): 16.
5. Gibbs, W Wayt. Untangling the roots of cancer. Scientific American. 2003; 57–65.
6. Conrotto P, Souchelnytskyi S. Proteomic approaches in biological and medical sciences: principles and applications. Experimental Oncology 2008; 30(3): 171–80.
7. Caretta A, Mucignat-Caretta C. Protein Kinase A in Cancer. Cancers 2011; 3; 913-926.
8. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat. Rev. Cancer. 2005; 5; 172–183.
9. Stork PJ, Schmitt JM. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. Trends Cell Biol. 2002; 12; 258–266.
10. Insel PA, Zhang L, Murray F, Yokouchi H, Zambon AC. Cyclic AMP is both a pro-apoptotic and antiapoptotic second messenger. Acta Physiol (Oxf). 2012; 204; 277-287.
11. Chiaradonna, F, Balestrieri, C, Gaglio, D, Vanoni, M. RAS and PKA pathways in cancer: New insight from transcriptional analysis. Front. Biosci. 2008; 13; 5257–5278.
12. Mantovani G, Bondioni S, Ferrero S, Gamba B, Ferrante E, Peverelli E et al. Effect of cyclic adenosine 3Œ§,5Œ§ -monophosphate/protein kinase A pathway on markers of cell proliferation in nonfunctioning pituitary adenomas. J. Clin. Endocrinol. Metab. 2005; 90; 6721–6724.
13. Lin T-C, Su C-Y, Wu P-Y, Lai T-C, Pan W-A, Jan Y-H et al. The nucleolar protein NIFK promotes cancer progression via CK1a/b-catenin in metastasis and Ki-67-dependent cell proliferation. eLife. 2016; 5; e11288.
14. Wang Z, Liu N, Shi S, Liu S, Lin H. The Role of PIWIL4, an Argonaute Family Protein, in Breast Cancer. The Journal of Biological Chemistry. 2016; 291; 10646-10658.
15. Chen H, Xu C, Jin Q, Liu Z. S100 protein family in human cancer. Am J Cancer Res. 2014; 4(2): 89-115.
16. Stevenson PR, Veltman D, Machesky ML. Actin-bundling proteins in cancer progression at a glance. Journal of Cell Science. 2012; 125; 1073–1079.
17. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst. 2000; 92; 1472–89.
18. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr. 2001; 131(suppl): S3109–20.
19. Renehan GA, Zwahlen M, Minder C, O'Dwyer TS, Shalet MS, Egger M. The Lancet. 2004; 363; 1346–53.
20. Wang Z, Liu P, Inuzuka H, Wei W. Roles of F-box proteins in cancer. Nat Rev Cancer. 2014; 14(4): 233–247.
21. Abhijnya KVV, Sreedhar AS. Heat Shock Proteins in the Cancer Immunity: Comprehensive Review on Potential Chemotherapeutic Interventions. J Clin Cell Immunol. 2012; S5; 1-8.
22. Chan S-L, Yu CV. Proteins of the Bcl-2 Family In Apoptosis Signalling: From Mechanistic Insights to Therapeutic Oppertunities. Clinical and Experimental Pharmacology and Physiology. 2004; 31; 119–128.
23. Stavraka C, Blagden S. The La-Related Proteins, a Family with Connections to Cancer. Biomolecules. 2015; 5; 2701-2722.
24. Tansey PW. Mammalian MYC Proteins and Cancer. New Journal of Science 2014; Article ID 757534, 27 pages.
25. Lipper HC, Paddock LM, Onuchic NJ, Mittler R, Nechushtai R, Jennings AP. Cancer Related NEET Proteins Transfer 2Fe2S Clusters to Anamorsin, a Protein Required for Cytosolic Iron-Sulfur Cluster Biogenesis, PlosOne. 2015; 1-15.
26. Jia Y., Zhou L, Tian C, Shi Y, Wang C, Tong Z. Dynamin-related protein 1 is involved in micheliolide-induced breast cancer cell death. OncoTargets and Therapy. 2015; 8; 3371–3381.

Review Article

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Potential of Dietary Polyphenols in Prevention and Treatment of Cancer

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Abstract

Polyphenols and flavonoids from natural products possess antioxidant properties which are known to prevent as well as treat cancer. Natural compounds from plant kingdom are non-toxic in a wide range of concentrations and are safe for long term usage. Many studies have demonstrated anticancer properties of many polyphenols and other antioxidants involving cell cycle modulation, transcription factor NF-κB and protein kinases. Research results from our laboratory have shown enhanced cytotoxic activity of a number of polyphenols, such as triphala, ellagic acid, curcumin, biochanin A and others in tumor cells in combination with radiation whereas normal cells were protected against the damage. The present paper gives a brief review of differential action of polyphenols in tumor and normal cells and outlines the mechanism of action through induction of apoptosis in oxidatively stressed cells. It is suggested that antioxidants drive cells to survival or death depending upon the level of homeostasis. Results point to clinical evaluation of some of these polyphenols for practical applications in cancer radiotherapy.

Keywords: Polyphenols; Cancer; Antioxidants; Oxidative Stress; Chemoprevention.

Introduction

There is increasing evidence to suggest that sedentary life styles, food habits, environmental changes produce adverse effects on human health [1-5]. Among them, stress related diseases like diabetes, coronary heart diseases and cancer pose major health issues to people around the world. It is reported that changing lifestyle in developing countries have worsened the situation of cancer prevalence [6]. Furthermore, recent reports suggest that obesity causes major risk factor for various types of cancer including oesophagus (adenocarcinoma), colo-rectum, breast (postmenopausal), endometrium and kidney [6-8].

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Research findings are in agreement that food habits and diet patterns are directly associated with cancer induction and progression. Pertinent to it, bioactive compounds from herbs and spices were investigated for disease prevention and management in wide range of concentrations which may exceed those commonly used in food preparations [9-15]. Reports have appeared suggesting that regular intakes of fruit, vegetables and whole grains are associated with reduced risk of chronic diseases including cancer [10,15,16]. Moreover, epidemiological studies suggest that high dietary intake of fruits, vegetables and whole grains are strongly associated with reduced risk of chronic diseases including cancer. WHO report of 2003 suggested convincing linkage between diet related factors and cancer prevalence. It has been found that fruits and vegetables showed preventive effect against cancers of oral cavity, esophagus, stomach and colorectum, while preserved and red

meat, salt-preserved foods and high salt intake, very hot drinks and foods probably increase the risk of colorectal, stomach, oral cavity, pharynx and oesophagus cancer respectively [17-18].The case control study conducted by Salem et al. (2011) suggests that total fat content increases risk of prostate cancer which was attenuated by the consumption of food rich in tomato and garlic content [19].

The therapeutic value of medicinal plant / herb lies in their bioactive compounds which possess specific physiological action in the human body. It is well established that herbs serve an important role in maintaining redox homeostasis that determines the fate of diseased cell. Furthermore, dietary antioxidants such as polyphenols, flavonoids, tannins derived from herbs and spices are known to prevent stress related adverse health consequences [13,20,21]. Polyphenols are secondary metabolites of plants, primarily occurring in conjugated forms with one or more hydroxyl (-OH) groups attached to benzene ring. Epidemiological studies on polyphenol consumption and human cancer risk suggest the protective effects of certain food items and polyphenols [13,22,23]. Polyphenols and other bioactive compounds are reported to exert their effects by scavenging oxygen free radicals and inhibiting the lipid peroxidation and protecting cellular macromolecules such as DNA from oxidative damage [11,12,14,24-26]. Commonly, polyphenols are recognized as naturally occurring antioxidants but they may act as pro-oxidants catalyzing DNA degradation in the presence of transition metal ions such as, iron and copper [27-29]. Antioxidants derived from herbs have potential to inhibit and / or influence pathways which regulate cell division, cell cycle proliferation, apoptosis and detoxification [11,15,21,30,31]. It has been reported that herbals like Triphala exhibit differential toxicity to normal and tumor cells [11,12]. The molecular mechanism is reported to involve differences in their ability to induce apoptosis in normal and tumor cells with and without irradiation. It is known that a number of antioxidants are safe across broad range of intakes; doses beyond tolerable limits of cellular environment serve as pro-oxidants to them. Culinary herbs and spices generally serve as antioxidants (AOs) but may serve as pro-oxidants at higher concentrations. It is important to be noted that herbs and spices are generally recognized as safe at their minimum effective concentration normally available in foods and herbal preparations.

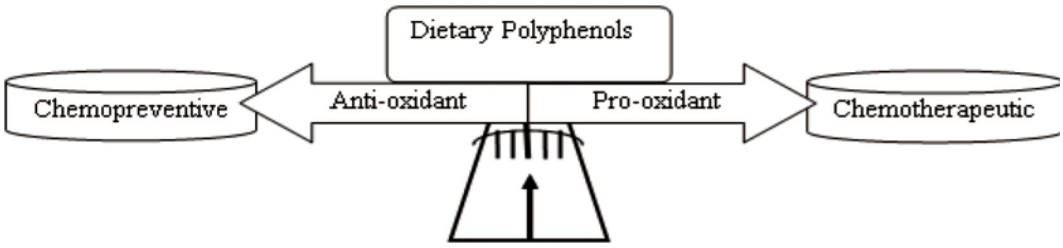

Fig. 1: Cartoon image depicting the delicate balance between anti-oxidant and pro-oxidant behavior of dietary polyphenols

Research findings especially reports from our group have demonstrated differential roles of many herbal antioxidants and their formulations in cellular studies [11,12,25,32-35]. The same drug that was involved in sensitization of tumor cells was found to be protective or inactive towards healthy cells together with radiation. It is to be noted that herbals possess pluripotent activity, display synergistic mode of action, produce selective effect on the target and also have low toxicity and are cost effective. Our publications have shown that many bioactive compounds of natural origin rich in polyphenols, flavonoids, and alkaloids possess radiosensitizing and radioprotective properties [11,12,33,34,36-41].

This review is aimed to summarize the chemo-preventive properties of some readily available dietary polyphenols such as gallic acid, curcumin, betulinic acid against cancer as observed in cell culture models. The major focus of the present review is to present the molecular mechanisms of chemo-preventive and therapeutic activities of dietary polyphenols with particular emphasis on their ability to control intracellular signaling cascade considered as relevant targets in a cancer preventive approach.

Experimental Studies and Proposed Mode of Action

Cancer cells are consequence of shutting down of built-in apoptosis and therefore, it is more relevant to search for compounds involved in regaining apoptosis mechanisms for bringing back normal cellular status. Results have shown that bioactive compounds and their derivatives present in different diets were involved in protection of normal cells as

antioxidant against oxidative damage. On the other hand, the same compound behaved as pro-oxidant inducing oxidative damage, membrane alterations, cell cycle arrest leading to apoptosis in transformed / cancer cells. Their molecular mechanism of action is reported to involve differences in their ability to induce apoptosis in normal and tumor cells with or without irradiation. AOs have ability to recognize the cytosolic redox status of cells which is known to be different in normal and tumor cells. As discussed earlier, antioxidants are found to upregulate endogenous defense machinery in normal cells and underlying mechanisms of action include cell cycle arrest, alterations in survival signaling, apoptosis and regulation of detoxifying enzymes.

In what follows we provide results of our studies on some polyphenols on normal and tumor cells in combination with gamma radiation. Also, references are cited from other studies where required. Results have allowed to conclude that differential action of polyphenol compounds on normal and tumor cells consist in the cytosolic redox status of respective cells. Involvement of signaling mechanisms is noticed in the manifestation of toxic cellular responses to drug or combinations of drug and radiation.

Triphala Enhances Tumor Radiotoxicity

Triphala (TPL) is an Ayurvedic herbal formulation consisting of equal proportions of three myrobalans, namely, Amalaki (*Emblica officinalis*), Bibhitaki (*Terminalia bellirica*), and Haritaki (*Terminalia chebula*). Results from our studies have demonstrated differential toxicities to normal and tumor cells. It was found that triphala along with gamma irradiation produced radio-sensitization to breast cancer cell lines *in vitro* [11,12]. It was further found that normal mouse hepatocytes and spleen cells were unaffected at concentrations of triphala that were toxic to the breast cancer cell line, MCF 7 . It was concluded that TPL enhanced radiotoxicity of cancer cell lines but spared normal cells primarily due to differences in the induced reactive oxygen species (ROS). More recently, Sharma et al. (2011) reported that TPL up-regulated glutathione in normal cells leading to prevention of stress mediated peroxidative damage in endoplasm [26]. Also, gallic acid (GA; 3,4,5-trihydroxybenzoic acid), an important stress buster antioxidant and anticancer agent is reported present in Triphala. In another study conducted by Russel and coworkers (2011), it was found that combined treatment of GA with flutamide induced greater toxicity to prostate cancer cells than either of the compounds alone with concomitant low toxicity to normal cells [32]. Lu et al.(2012) have reported that chebulinic (CI) present in TPL can significantly and specifically inhibit vascular endothelial growth factor-A (VEGF) induced angiogenesis by suppressing VEGF receptor-2 (VEGFR-2) phosphorylation [43].

Ellagic Acid Sensitizes Tumor Cells to Radiation

Natural antioxidant, Ellagic Acid (EA) is abundant in berry and nut fruits like strawberries, raspberries, wolfberries, grapes ,walnuts, pomegranate [44], oak-aged red wine, peach and other plant foods.

Extensive studies were carried out in our laboratory on the combined effects of radiation and ellagic acid (EA) both *in vitro* and *in vivo* on normal and tumor cells. Interestingly, human cervical cell line have shown increased ROS generation as a function of radiation dose [37]. Ellagic acid mediated increased cytotoxicity of tumor cells was found associated with the increased intracellular ROS level. In contrast, EA protected significantly the normal splenic lymphocytes against radiation- induced oxidative stress. *In vitro* and *in vivo* studies have further demonstrated anti-cancer activity on many cancer cells such as cervical, oesophagus, breast, colon, prostate and pancreas [45-53]. It was suggested that EA arrested cell-cycle in S phase, stimulated apoptosis via FAS-independent and caspase 8-independent pathway in human colon cancer cell line and protected DNA damage in normal colon cells line [45]; induce G0 / G1 arrest, promoted ROS and Ca^{2+} production [51]. It possess the ability to reduce endogenous oxidative DNA damage by DNA excision repair protein (ERCC5) and DNA ligase III (DNL3) [46]. In recent studies, EA mediated apoptosis via mitochondrial pathway in human neuroblastoma cell line was suggested to be dose and time-dependent [52]. Vanella et al. (2013) showed antiproliferative and pro-differentiation properties of EA inducing DNA damage in cancer cells [49,50]. EA is reported to stimulate apoptosis and decrease proliferation in human pancreatic adenocarcinoma cells through DNA fragmentation, mitochondrial depolarization, release of cytochrome, and the downstream caspase activation in pancreatic cancer cells. Furthermore, it is reported to block the NF-κB binding activity in dose-dependent manner [47]. Malik and coworkers (2011) reported dose-dependent inhibition of cell growth and apoptosis in human prostate cancer cells. Underlying mechanism involved cleavage of poly (ADP-ribose) polymerase (PARP), decreased levels of anti-apoptotic protein Bcl-2 and up-regulated pro apoptotic protein Bax [48]. Recent investigation from our research group showed that EA together with

gamma radiation induced apoptosis via upregulation of ROS, calcium levels and caspase-3 activity resulting in decreased mitochondiral potential [53].

Eugenol Acts as Prooxidant as Well as Antioxidant

Eugenol (4-Allyl-2-methoxyphenol) is an active component of Indian medicinal plants, clove (*Syzygium aromaticum*) , tulsi (*Oscimum sanctum*) and other aromatic plants like cinnamon, bay leaves. It has been demonstrated that modulation of phytochemical properties of model as well as cellular membranes by inclusion of antioxidants like eugenol caused inhibition of membrane oxidative damage. Results from our studies demonstrated significant enhanced bilayer rigidity in irradiated phoshpholipids liposomal as model membrane due to free radical mediated reaction of lipoxy radicals [36,38].

It was found that eugenol displays both prooxidant and antioxidant activities at different doses. It enhanced H_2O_2 induced cytotoxicity resulting in damage to cell membrane and DNA in resistant cancer cell line [35]. The anti-cancer and chemopreventive mechanisms of eugenol involved decreased glutathione level and increased lipid peroxidation in breast cancer cells accompanied with cell shrinkage, membrane blebbing , intracellular non protein thiol depletion and induces apoptosis via DNA fragmentation [54,55]. Arif et al.(2011) demonstrated strong synergistic interaction between eugenol- gemcitabine, which may enhance the therapeutic index of prevention and / or treatment of cervical cancer [21]. Their results suggest that eugenol exerts its anticancer activities *via* induction of apoptosis and anti-inflammatory properties with significant downregulation of Bcl-2, COX-2, and IL-1β on treatment with eugenol [21].

Curcumin Acts as Tumor Radiosensitizer

Turmeric (*Curcuma longa*) is one of the most popular dietary ingredient of Indians. Curcumin (1,7-bis (4-hydroxy 3-methoxy phenyl)-1,6-heptadiene- 3,5-dione) is one of three curcuminoids of turmeric, a highly promising natural antioxidant with multiple mechanisms to prevent cancer [56]. Curcumin modulates multiple molecular pathways involved in the complex carcinogenesis process to exert its chemopreventive effects through several mechanisms: promoting apoptosis, inhibiting survival signals, scavenging reactive oxidative species (ROS), and reducing the inflammatory cancer microenvironment [56-58]. This polyphenol acts as a radiosensitizer – in prostate cancer by down regulation of pro-survival factors. Studies in our laboratory showed that exposure of phenolic compound curcumin prior to irradiation decreased breast cancer cell (MCF-7) survival to 38% as compared to 52% by radiation alone. Interestingly, treatment of MCF-7 cells with curcumin caused significant enhancement of gamma radiation-induced cell death, potentially mediated via ROS independent pathway [39]. Studies have proven that various bioactive components of turmeric sensitize tumor cells towards radiation exposure by upregulating apoptotic gene with simultaneous downregualtion of survival factors like NF-kB, Cox-2, Akt, STAT3, anti-apoptotic and multidrug resistant proteins [25,59-64].

Nigella Sativa (Ns) *Protects Cells Against Oxidative Damage*

Common Asian spice *Nigella sativa* (Black Cumin) also known as black seed, kalonji appears to be effective at doses used to season food products. The results from our laboratory showed that the macerated extract of NS seeds protected the liver, spleen, brain and intestines both in normal as well as tumor bearing mice [33-34]. This study concludes that macerated extract of NS seeds has protective effects against radiation-induced damage and biochemical alterations which could be attributed to the ability to scavenge free radicals and its antioxidant properties. The results obtained from the different experimental systems suggest the radioprotective ability of ethanol extract NS involving prevention of radiation-induced oxidative damage [20]. Furthermore, significant free radical scavenging and protection against DNA damage in cell free systems was found. Parallel investigations by other researchers demonstrated significant anti-cancer activities against a number of cancer cells such as breast cancer, hepatic, cervical squamous carcinoma cells, hence support our findings [65-69]. Alenzi et al. (2010) demonstrated up-regulation of antioxidant, indicated a potential clinical application to minimize the toxic effects of treatment with anticancer drugs [65]. Majdalawieh and co-workers demonstrated modulating effect of NS seeds in splenocyte proliferation, Th1 / Th2 cytokine profile, macrophage function and NK anti-tumor activity [66]. Ng et al. (2011) showed that thymoquinone from *N. sativa* efficiently eliminated SiHa cells via apoptosis with down-regulation of Bcl-2 protein [67].

Cytoprotection and Cytotoxic Activity of Helicteres Isora (HI)

H. isora is a tropical south-east Asian shrub available throughout India. The twisted shape of the

fruit resembles that of a deer's horn. Significant presence of polyphenols in fruits of HI [70], though hardly been investigated for chemotherapeutic property prompted us to investigate anticancer activity of fruits (results under communication). Aqueous and alcoholic extracts from fruits and bark of HI are reported to display antioxidant activity such as free radical scavenging [70-73]. Pradhan et al.(2008) demonstrated cytoprotective role of methanolic extract of fruits of HI alongwith antitumor activity [72]. Raman et al. (2012) reported anticancer activity and presence of antioxidants in acetone extract of fruits of HI [73].

Tumor Radiosensitization by Biochanin A

Biochanin A (BCA; 5,7-Dihydroxy-4'-methoxyis oflavone) is a major dietary isoflavone of soy cabbage, alfalfa and red clover, that possess chemopreventive properties. One of our new study describes positive radiosensitizing effect of flavonoid, BCA, on the growth of radioresistant human colon cancer HT29 cells *in vitro*. We found that combined treatment yielded an additive increase of caspase-3 in these radioresistant colon cells. Treatment combined with irradiation caused significant decrease of cell proliferation along with substantial increase of ROS, lipid peroxidation and mitochondrial membrane potential. Furthermore, it was also found that combined treatment yielded an additive increase of caspase-3 in these radioresistant colon cells [41]. Recent investigations by many other researchers have shown positive results of BCA against drug resistant factors of prostate , pancreatic, breast cancer cells. BCA overcame Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistance by engaging both intrinsic and extrinsic apoptotic pathways and by regulating the NF-êB activity [74]. Sehdev et al.(2010) found that BCA can selectively target cancer cells and inhibit multiple signaling pathways in HER-2-positive breast cancer cells [75]. Kole at al.(2011) have shown that anti-proliferative and anti-inflammatory activities of BCA was mediated by the inhibition of iNOS expression, p38-MAPK and ATF-2 phosphorylation and blocking NF-kB nuclear translocation [76].

Moreover, BCA is found effective in reducing pancreatic cancer cell survival by inhibiting their proliferation and inducing apoptosis by reducing their colony formation ability via dose-dependent apoptosis and inhibiting activation of Akt and MAPK [77].

Anti Cancer Effects of Betulinic Acid

Betulinic acid (BA; 3β, hydroxy-lup-20(29)-en-28-oic acid), a natural triterpene abundant in the outer bark of white birch trees, *Betula alba* L and other tree species . There are many evidences that indicate BA as a potent antioxidant source possessing anti-cancer activity . According to Simone Fulda, mitochondrion-targeted agents such as betulinic acid hold great promise as a novel therapeutic strategy in the treatment of human cancers [78]. Betulinic acid is reported to inhibit colon cancer cell and tumor growth and induces proteasome-dependent and-independent down regulation of specificity proteins (Sp) transcription factors[79]. In one of their recent investigations Mertens-Talcott and co-workers reported that BA decreases ER-negative breast cancer cell growth *in vitro* and *in vivo*. This anticancer effect of BA was at least in part based on interactions with the microRNA-27a-ZBTB10-Sp-axis causing increased cell death [80]. In one of our recent investigations positive cytotoxic effect of BA on breast cancer cell lines was observed [42]. Our results have shown that anti-tumor activity of BA is not restricted to melanoma and neuroectodermal tumors but it also causes cytotoxicity in breast cancer cells. Treatment MCF-7 and T47D cell lines with BA resulted in a dose dependent inhibition of cell proliferation and induction of p53 independent apoptosis. Furthermore, the induction of apoptosis also showed an alteration in membrane permeability, encompassing the role of membrane damage in BA induced apoptosis [42]. In another report by Yi et al. (2014) hepatoprotective role of BA was demonstrated with improved tissue redox system and decreased lipid peroxidation [81].

Perspectives on Polyphenols with Relevance to Cancer Therapy

Development of diet-derived constituents is one of the major goal in prevention of stress related diseases such as for cancer chemoprevention, cardiovascular disease, diabetes. Various studies have suggested that dietary polyphenols are more than just antioxidants. They have multiple biological functions including anticancer effects. A popular belief is that dietary polyphenols possess anticancer property since they are antioxidants that are free radical scavengers and regulate redox balance essential to maintain appropriate balance between cell proliferation and death. They are recognized as naturally occurring antioxidants but may act as pro-oxidants catalyzing DNA degradation in the presence of transition metal ions such as copper [27-29]. The ability to generate ROS or binding and cleavage of DNA by dietary polyphenols in presence of transition metal ions are similar to many conventional anticancer drugs [21,82,83]. A well

recognized anticancer activity of dietary polyphenols is DNA fragmentation mediated apoptosis. It is assumed involve mobilization of intra- and extra-cellular copper [30]. A report from Khan et al.(2012) suggested DNA cleavage by resveratrol and Cu^{2+}, where it was found that resveratrol forms a complex with Cu^{2+} reducing it to Cu^{1+} with formation of another oxidized species of resveratrol [30].

Polyphenols inhibit cell growth, by inducing cell cycle arrest and / or apoptosis; inhibit proliferation, differentiation, inflammation, angiogenesis, and / or metastasis; and exhibit anti-inflammatory and / or antioxidant effects [14,31,56,84]. Moreover, as chemopreventive agents, polyphenols have been reported to hinder with cancer initiation, promotion and progression [14,15, 56]. Henceforth, the probable mode of cytoprotection and mechanisms associated with their biological effects includes (i) antioxidant and free radical scavenging activity (ii) trapping of activated metabolites of carcinogens (iii) prevention of mutagenicity and genotoxicity (iv) Inhibition of biochemical markers of tumor initiation and promotion (v) Differential role of detoxification enzymes. Our previous research findings are accordance with the proposed hypothesis. Increasing body of scientific evidence developed over the years has demonstrated the potential role of antioxidants in both prevention of healthy cells and killing of diseased cells.

Taken together, it can be said that, dietary polyphenols as antioxidants usually functions in protective mode. Plant polyphenols are important components of human diet and as antioxidants a number of them are considered to possess therapeutic property against many types of cancer. In recent years, a large number of studies have attributed a protective effect to natural food, herbs and spices containing these compounds against cancer and other stress related diseases [13]. Epidemiological studies concerning polyphenol consumption and human cancer risk suggest the protective effects of certain food items and polyphenols [13,22,23]. The pro-oxidant activity in curative mode activity is most prominent under *in vitro* conditions such as at a high pH in the presence of high concentrations of transition metal ions and oxygen molecules. They possess pro-oxidant activities both *in vitro* and *in vivo*, that may contribute to some of their biological properties such as antioxidant, pro-oxidant and anticancer effects [13,22,84]. Reports have shown that the phenol ring (ring B- in particular) of polyphenols causes free radical generation, oxidation of endogenous enzymes such as glutathione [21,24,85]. For instance, dietary polyphenol quercetin contains a catechol B ring that is oxidized by peroxidases to quinone with subsequent reaction with glutathione forming quercetin glutathionyl byproducts [24,85].

Conclusion

We have shown that several herbal compounds in combination with ionizing radiation enhance tumor cell killing and act as radiosensitizers while leave normal cells unaffected or even cause protection to them. It is suggestive that the differential behavior of the studied bioactive compounds is due to multiple mode of actions at different cellular targets under different cyotosolic status. It has been found that cytotoxic action was induced by upregulating reactive oxygen species thus disturbing the antioxidant status of cancer cells. The data provided herein provides a good scientific rationale for undertaking clinical trials of above mentioned herbal drugs. Reasonable amount of these plants and their byproducts are safe across a broad range of dose limits and can be consumed as dietary supplements.

Conflict of Interest

Declared None

References

1. Otero-Sabogal R, Sabogal F, Perez-Stable EJ, Hiatt RA. Dietary practices, alcohol consumption, and smoking behavior: ethnic, sex, and acculturation differences. Journal of the National Cancer Institute. Monographs. 1994 Dec; (18): 73-82.
2. Wang JM, Xu B, Rao JY, Shen HB, Xue HC, Jiang QW. Diet habits, alcohol drinking, tobacco smoking, green tea drinking, and the risk of esophageal squamous cell carcinoma in the Chinese population. European journal of gastroenterology & hepatology. 2007 Feb 1; 19(2): 171-6.
3. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N. Physiological and health implications of a sedentary lifestyle. Applied Physiology, Nutrition, and Metabolism. 2010 Nov 23; 35(6): 725-40.
4. Thorp AA, Owen N, Neuhaus M, Dunstan DW. Sedentary behaviors and subsequent health outcomes in adults: a systematic review of longitudinal studies, 1996–2011. American journal of preventive medicine. 2011 Aug 31; 41(2): 207-15.
5. Vilchis-Gil J, Galván-Portillo M, Klünder-Klünder M, Cruz M, Flores-Huerta S. Food habits, physical activities and sedentary lifestyles of eutrophic and obese school children: a case–control study. BMC

public health. 2015 Feb 11; 15(1): 1.

6. McCormack VA, Boffetta P. Today's lifestyles, tomorrow's cancers: trends in lifestyle risk factors for cancer in low-and middle-income countries. Annals of Oncology. 2011 Nov 1; 22(11): 2349-57.
7. Key TJ, Schatzkin A, Willett WC, Allen NE, Spencer EA, Travis RC. Diet, nutrition and the prevention of cancer. Public health nutrition. 2004 Feb 1; 7(1a): 187-200.
8. Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. Annals of the New York Academy of Sciences. 2012 Oct 1; 1271(1): 37-43.
9. Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW. Klein S, Rister RS (trans) The Complete German Commission E Monographs—Therapeutic Guide to Herbal Medicines. American Botanical Council, Austin, Texas. Integrative Medicine Communication, Boston, MA.: 36-138. 1998.
10. Kaefer CM, Milner JA. The role of herbs and spices in cancer prevention. The Journal of nutritional biochemistry. 2008 Jun 30; 19(6): 347-61.
11. Sandhya T, Mishra KP. Cytotoxic response of breast cancer cell lines, MCF 7 and T 47 D to triphala and its modification by antioxidants. Cancer letters. 2006 Jul 18; 238(2): 304-13.
12. Sandhya T, Lathika KM, Pandey BN, Bhilwade HN, Chaubey RC, Priyadarsini KI, et al. Protection against radiation oxidative damage in mice by Triphala. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2006; 609(1): 17-25.
13. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition journal. 2010 Jan 22; 9(1): 1.
14. Araújo JR, Gonçalves P, Martel F. Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutrition Research. 2011 Feb 28; 31(2): 77-87.
15. Lewandowska H, Kalinowska M, Lewandowski W, Stêpkowski TM, Brzóska K. The role of natural polyphenols in cell signaling and cytoprotection against cancer development. The Journal of nutritional biochemistry. 2016 Jun 30; 32: 1-9.
16. La Vecchia C, Tavani A. Fruit and vegetables, and human cancer. European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation *(ECP)*. 1998 Feb; 7(1): 3-8.
17. Tsugane S. [Dietary factor and cancer risk—evidence from epidemiological studies]. Gan to kagaku ryoho. Cancer & chemotherapy. 2004 Jun;31(6):847-52.
18. Etemadi A, Golozar A, Kamangar F, Freedman ND, Shakeri R, Matthews C, et al. Large body size and sedentary lifestyle during childhood and early adulthood and esophageal squamous cell carcinoma in a high-risk population. Annals of oncology. 2011 Nov 2: mdr494.
19. Salem S, Salahi M, Mohseni M, Ahmadi H, Mehrsai A, Jahani Y, Pourmand G. Major dietary factors and prostate cancer risk: a prospective multicenter case-control study. Nutrition and cancer. 2011 Jan 10; 63(1): 21-7.
20. Rastogi L, Feroz S, Pandey BN, Jagtap A, Mishra KP. Protection against radiation-induced oxidative damage by an ethanolic extract of Nigella sativa L. International journal of radiation biology. 2010 Sep 1; 86(9): 719-31.
21. Arif H, Rehmani N, Farhan M, Ahmad A, Hadi SM. Mobilization of Copper ions by Flavonoids in Human Peripheral Lymphocytes Leads to Oxidative DNA Breakage: A Structure Activity Study. International journal of molecular sciences. 2015 Nov 9; 16(11): 26754-69.
22. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual review of nutrition. 2001 Jul; 21(1): 381-406.
23. Sun CL, Yuan JM, Koh WP, Mimi CY. Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. Carcinogenesis. 2006 Jul 1; 27(7): 1310-5.
24. Galati G, O'Brien PJ. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radical Biology and Medicine. 2004 Aug 1; 37(3): 287-303.
25. Goel A, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. Nutrition and cancer. 2010 Sep 23; 62(7): 919-30.
26. Sharma A, Sharma KK. Chemoprotective role of triphala against 1, 2-dimethylhydrazine dihydrochloride induced carcinogenic damage to mouse liver. Indian Journal of Clinical Biochemistry. 2011; 26(3): 290-95.
27. Hadi SM, Bhat SH, Azmi AS, Hanif S, Shamim U, Ullah MF. Oxidative breakage of cellular DNA by plant polyphenols: a putative mechanism for anticancer properties. In Seminars in cancer biology 2007 Oct 31; 17(5): 370-376). Academic Press.
28. Hadi SM, Ullah MF, Shamim U, Bhatt SH, Azmi AS. Catalytic therapy of cancer by ascorbic acid involves redox cycling of exogenous/endogenous copper ions and generation of reactive oxygen species. Chemotherapy. 2010 Aug 11; 56(4): 280-4.
29. Gupte A, Mumper RJ. Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. Cancer treatment reviews. 2009 Feb 28; 35(1): 32-46.
30. Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer research. 2006 Mar 1 ;66(5): 2500-5.

31. Ramos S. Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. Molecular nutrition & food research. 2008 May 1; 52(5): 507-26.

32. Russell LH, Mazzio E, Badisa RB, Zhu ZP, Agharahimi M, Millington DJ, Goodman CB. Differential cytotoxicity of triphala and its phenolic constituent gallic acid on human prostate cancer LNCap and normal cells. Anticancer research. 2011 Nov 1; 31(11): 3739-45.

33. Velho Pereira R, Kumar A, Pandey BN, Jagtap AG, Mishra KP. Radiosensitization in human breast carcinoma cells by thymoquinone: role of cell cycle and apoptosis. Cell biology international. 2011 Oct 1; 35(10): 1025-9.

34. Velho-Pereira R, Kumar A, Pandey BN, Mishra KP, Jagtap AG. Radioprotection by macerated extract of Nigella sativa in normal tissues of fibrosarcoma bearing mice. Indian journal of pharmaceutical sciences. 2012 Sep; 74(5): 403.

35. Özkan A, Erdoğan A. Membrane and DNA damaging / protective effects of eugenol, eucalyptol, terpinen-4-ol, and camphor at various concentrations on parental and drug-resistant H1299 cells. Turkish Journal of Biology. 2013 Aug 20; 37(4): 405-13.

36. Pandey BN, Mishra KP. Modification of thymocytes membrane radiooxidative damage and apoptosis by eugenol. Journal of environmental pathology, toxicology and oncology. 2004; 23(2).

37. Bhosle SM, Huilgol NG, Mishra KP. Enhancement of radiation-induced oxidative stress and cytotoxicity in tumor cells by ellagic acid. Clinica chimica acta. 2005 Sep 30; 359(1): 89-100.

38. Pandey BN, Lathika KM, Mishra KP. Modification of radiation-induced oxidative damage in liposomal and microsomal membrane by eugenol. Radiation Physics and Chemistry. 2006 Mar 31; 75(3): 384-91.

39. Girdhani S, Ahmed M, Mishra KP. Enhancement of gamma radiation-induced cytotoxicity of breast cancer cells by curcumin. Mol. Cell Pharmacol. 2009 Aug 10; 1: 208-17.

40. Rastogi L, Feroz S, Pandey BN, Jagtap A, Mishra KP . Protection against radiation-induced oxidative damage by an ethanolic extract of Nigella sativa L. International Journal of Radiation Biology. 2010 Sep1; 86(9): 719-31.

41. Puthli A, Tiwari R, Mishra KP. Biochanin A enhances the radiotoxicity in colon tumor cells in vitro. Journal of Environmental Pathology, Toxicology and Oncology. 2013; 32(3).

42. Tiwari R, Puthli A, Balakrishnan S, Sapra BK, Mishra KP. Betulinic acid-induced cytotoxicity in human breast tumor cell lines MCF-7 and T47D and its modification by tocopherol. Cancer investigation. 2014 Sep 14; 32(8): 402-8.

43. Lu K, Chakroborty D, Sarkar C, Lu T, Xie Z, Liu Z, Basu S. Triphala and its active constituent chebulinic acid are natural inhibitors of vascular endothelial growth factor-a mediated angiogenesis. PLoS One. 2012 Aug 24; 7(8): e43934.

44. Vattem DA, Shetty K. "Biological Function of Ellagic Acid: A Review". Journal of Food Biochemistry. 2005; 29(3): 234–66.

45. Larrosa M, Tomás-Barberán FA, Espín JC. The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. The Journal of nutritional biochemistry. 2006 Sep 30; 17(9): 611-25.

46. Aiyer HS, Vadhanam MV, Stoyanova R, Caprio GD, Clapper ML, Gupta RC. Dietary berries and ellagic acid prevent oxidative DNA damage and modulate expression of DNA repair genes. International journal of molecular sciences. 2008 Mar 12; 9(3): 327-41.

47. Edderkaoui M, Odinokova I, Ohno I, Gukovsky I, Go VL, Pandol SJ, Gukovskaya AS. Ellagic acid induces apoptosis through inhibition of nuclear factor κB in pancreatic cancer cells.

48. Malik A, Afaq S, Shahid M, Akhtar K, Assiri A. Influence of ellagic acid on prostate cancer cell proliferation: A caspase–dependent pathway. Asian Pacific journal of tropical medicine. 2011 Jul 31; 4(7): 550-5.

49. Vanella L, Barbagallo I, Acquaviva R, Di Giacomo C, Cardile V, G Abraham N, Sorrenti V. Ellagic acid: Cytodifferentiating and antiproliferative effects in human prostatic cancer cell lines. Current pharmaceutical design. 2013 May 1; 19(15): 2728-36.

50. Vanella L, Di Giacomo C, Acquaviva R, Barbagallo I, Li Volti G, Cardile V, Abraham NG, Sorrenti V. Effects of ellagic acid on angiogenic factors in prostate cancer cells. Cancers. 2013 Jun 19; 5(2): 726-38.

51. Ho CC, Huang AC, Yu CS, Lien JC, Wu SH, Huang YP, Huang HY, Kuo JH, Liao WY, Yang JS, Chen PY. Ellagic acid induces apoptosis in TSGH8301 human bladder cancer cells through the endoplasmic reticulum stress and mitochondria dependent signaling pathways. Environmental toxicology. 2014 Nov 1; 29(11): 1262-74.

52. Alfredsson CF, Ding M, Liang QL, Sundström BE, Nånberg E. Ellagic acid induces a dose-and time-dependent depolarization of mitochondria and activation of caspase-9 and-3 in human neuroblastoma cells. Biomedicine & Pharmacotherapy. 2014 Feb 28; 68(1): 129-35.

53. Ahire VR, Mishra KP, Kulkarni GR. Apoptotic Radiosensitivity of Cervical Tumor Cells Enhanced by Ellagic Acid. European Journal of Biotechnology and Bioscience. 2015 April; 3(4): 56-8.

54. Jaganathan SK, Mazumdar A, Mondhe D, Mandal M. Apoptotic effect of eugenol in human colon cancer cell lines. Cell biology international. 2011 Jun 1; 35(6): 607-15.

55. Vidhya N, Devaraj SN. Induction of apoptosis by

eugenol in human breast cancer cells. Indian Journal of Experimental Biology. 2011 Nov; 49: 871-8.

56. Prasad S, Gupta SC, Tyagi AK, Aggarwal BB. Curcumin, a component of golden spice: from bedside to bench and back. Biotechnology advances. 2014 Nov 1; 32(6): 1053-64.
57. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res. 2003; 23: 363-98.
58. Park W, Amin AR, Chen ZG, Shin DM. New perspectives of curcumin in cancer prevention. Cancer prevention research. 2013 May 1; 6(5): 387-400.
59. Sandur SK, Pandey MK, Sung B, Ahn KS, Murakami A, Sethi G, et al. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis. 2007 Aug 1; 28(8): 1765-73.
60. Jagetia GC, Aggarwal BB. "Spicing up" of the immune system by curcumin. Journal of clinical immunology. 2007 Jan 1; 27(1): 19-35.
61. Javvadi P, Hertan L, Kosoff R, Datta T, Kolev J, Mick R, Tuttle SW, Koumenis C. Thioredoxin reductase-1 mediates curcumin-induced radiosensitization of squamous carcinoma cells. Cancer research. 2010 Mar 1; 70(5): 1941-50.
62. Nada AS, Hawas AM, Amin NE, Elnashar MM, Abd Elmageed ZY. Radioprotective effect of Curcuma longa extract on ã-irradiation-induced oxidative stress in rats. Canadian journal of physiology and pharmacology. 2012 Mar 20; 90(4): 415-23.
63. Chiablaem K, Lirdprapamongkol K, Keeratichamroen S, Surarit R, Svasti J. Curcumin suppresses vasculogenic mimicry capacity of hepatocellular carcinoma cells through STAT3 and PI3K / AKT inhibition. Anticancer research. 2014 Apr 1; 34(4): 1857-64.
64. Chung SS, Vadgama JV. Curcumin and Epigallocatechin Gallate Inhibit the Cancer Stem Cell Phenotype via Down-regulation of STAT3–NFêB Signaling. Anticancer research. 2015 Jan 1; 35(1): 39-46.
65. Alenzi FQ, El-Bolkiny YE, Salem ML. Protective effects of Nigella sativa oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. British journal of biomedical science. 2010 Jan 1; 67(1): 20-8.
66. Majdalawieh AF, Hmaidan R, Carr RI. Nigella sativa modulates splenocyte proliferation, Th1 / Th2 cytokine profile, macrophage function and NK anti-tumor activity. Journal of ethnopharmacology. 2010 Sep 15; 131(2): 268-75.
67. Ng WK, Yazan LS, Ismail M. Thymoquinone from Nigella sativa was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. Toxicology in vitro. 2011 Oct 31; 25(7): 1392-8.
68. Mahmoud SS, Torchilin VP. Hormetic / cytotoxic effects of Nigella sativa seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. Cell biochemistry and biophysics. 2013 Jul 1; 66(3): 451-60.
69. Bahramian S, Bigdeli MR, Rasoulian B, Farimani MM. Antitumor and Antiangiogenic Effects of the Crude Oil of Nigella sativa L. in Tumor Tissues in BALB / c Mice. Zahedan Journal of Research in Medical Sciences. 2016 (In Press).
70. Jain A, Sinha P, Desai NS. Estimation of flavonoid, phenol content and antioxidant potential of Indian screw tree (*Helicteres isora* L.). International Journal of Pharmaceutical Sciences and Research. 2014 Apr 1; 5(4): 1320-30.
71. Pradhan M, Sribhuwaneswari S, Karthikeyan D, Minz S, Sure P, Chandu AN et al. In-vitro cytoprotection activity of Foeniculum vulgare and Helicteres isora in cultured human blood lymphocytes and antitumour activity against B16F10 Melanoma cell line. Research J. Pharm. and Tech. 2008; 1(4): 450-52.
72. Raaman N, Balasubramanian K, et al. Antioxidant and anticancer activity of Helicteres isora dried fruit solvent extracts. J Acad Indus Res. 2012; 1(3): 148-52.
73. Dayal R, Singh A, Ojha RP, Mishra K.P. Possible therapeutic potential of Helicteres isora (l.) and it's mechanism of action in diseases. Journal of Medicinal Plants Studies. 2015 Mar 03; 3(2): 95-100.
74. Szliszka E, Czuba ZP, Mertas A, Paradysz A, Krol W. The dietary isoflavone biochanin-A sensitizes prostate cancer cells to TRAIL-induced apoptosis. InUrologic Oncology: Seminars and Original Investigations. 2013 Apr 30; 31(3): 331-342. Elsevier.
75. Sehdev V, Lai JC, Bhushan A. Biochanin a modulates cell viability, invasion, and growth promoting signaling pathways in HER-2-positive breast cancer cells. Journal of oncology. 2010 Feb 11; 2009.
76. Kole L, Giri B, Manna SK, Pal B, Ghosh S. Biochanin-A, an isoflavon, showed anti-proliferative and anti-inflammatory activities through the inhibition of iNOS expression, p38-MAPK and ATF-2 phosphorylation and blocking NFêB nuclear translocation. European journal of pharmacology. 2011 Feb 25; 653(1): 8-15.
77. Bhardwaj V, Tadinada SM, Jain A, Sehdev V, Daniels CK, Lai JC, Bhushan A. Biochanin A reduces pancreatic cancer survival and progression. Anti-cancer drugs. 2014 Mar 1; 25(3): 296-302.
78. Fulda S. Betulinic acid for cancer treatment and prevention. International journal of molecular sciences. 2008 Jun 27; 9(6): 1096-107.
79. Chintharlapalli S, Papineni S, Lei P, Pathi S, Safe S. Betulinic acid inhibits colon cancer cell and tumor growth and induces proteasome-dependent and-

independent downregulation of specificity proteins (Sp) transcription factors. BMC cancer. 2011 Aug 24; 11(1): 1.

80. Mertens Talcott SU, Noratto GD, Li X, Angel Morales G, Bertoldi MC, Safe S. Betulinic acid decreases ER negative breast cancer cell growth in vitro and in vivo: Role of Sp transcription factors and microRNA 27a: ZBTB10. Molecular carcinogenesis. 2013 Aug 1; 52(8): 591-602.
81. Yi J, Xia W, Wu J, Yuan L, Wu J, Tu D, et al. Betulinic acid prevents alcohol-induced liver damage by improving the antioxidant system in mice. J Vet Sci. 2014; 15: 141–148.
82. Mohan A, Narayanan S, Sethuraman S, Maheswari Krishnan U. Combinations of plant polyphenols & anti-cancer molecules: a novel treatment strategy for cancer chemotherapy. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2013 Feb 1; 13(2): 281-95.
83. Rodríguez-Mercado JJ, Florín-Ramírez D, Álvarez-Barrera L, Altamirano-Lozano MA. In vitro DNA damage by Casiopeina II-gly in human blood cells. Drug and Chemical Toxicology. 2016 Jun 9: 1-7.
84. Marina Lecci R, Logrieco A, Leone A. Pro-oxidative action of polyphenols as action mechanism for their pro-apoptotic activity. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2014 Dec 1; 14(10): 1363-75.
85. Pereira RB, Sousa C, Costa A, Andrade PB, Valentão P. Glutathione and the antioxidant potential of binary mixtures with flavonoids: synergisms and antagonisms. Molecules. 2013 Jul 25; 18(8): 8858-72.

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[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

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[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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