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Supplementation of Taurine Alleviates Oxidative Stress and Dyslipidaemia in Streptozotacin Induced Diabetic Rats

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Abstract

Context: Diabetes mellitus (DM) is a metabolic disorder characterised by chronic hyperglycaemia, oxidative stress and dyslipidaemia. Glycaemic control may not always normalize the dyslipidaemia and antioxidant status. *Aim:* To study the effect of taurine on glycaemic control, antioxidant status and dislipidaemia in streptozotocin (STZ) induced diabetic rats. *Settings and Design:* It is an experimental study done on Wistar rats. *Materials and Methods:* Thirty two Wistar male albino rats of 19±1 weeks of age weighing 200-220 grams were randomly divided into four groups and each group consisted of eight animals. Group I (control) standard chow diet; Group II (chow diet plus taurine) Group III (diabetes induced), Group IV (diabetic receiving taurine). At the end of 45th day, all animals were sacrificed by cervical decapitation after overnight fasting. Blood and liver tissue samples were collected for analyses of plasma glucose, plasma and hepatic thiobarbituric acid reactive substances (TBARS), lipid profile, activities of lipoprotein lipase (LPL) and Lecithin cholesterol acyl transferase (LCAT) and histopathological studies of liver for fatty changes. *Statistical analysis used:* One way analysis of variance (ANOVA) test was applied in order to evaluate any significant difference in the mean values. *Results:* The present study found that there was no significant change in plasma glucose levels and plasma and hepatic TBARS levels were significantly reduced while a significant positive modulation of lipid profile and reduction of hepatic fats in diabetic rats treated with taurine. *Conclusions:* The present study indicates that taurine supplementation might be beneficial in alleviating oxidative stress and dyslipidaemia in diabetes.

Keywords: Diabetes; Streptozotacin; Taurine; Antioxidant; Dyslipidaemia

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterised by chronic hyperglycaemia with derangement in carbohydrate, fat and protein metabolism arising from a defect in insulin secretion or action or both [1]. Based on the aetiology and pathogenesis; DM is classified into two types: type 1 and type 2 DM [2]. The risk of developing diabetic complications is associated with age, duration of diabetes and this is greater in young diabetic patients [3]. Chronic hyperglycaemia causes cellular damage through several pathways such as increased non enzymatic glycation and increased lipid peroxidation and reduced antioxidant status, which result in over expression of gene products causing cellular damage [4, 5]. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins [6]. Oxidative stress due to generation of free radicals and accumulated products of lipid peroxidation plays a major role in the development of complications in diabetes [7].

Diabetes, especially type 2 is often associated with disorders in lipid metabolism [8]. Diabetic dyslipidaemia in subjects with type 2 commonly consists of elevated levels of triacylglycerol (TGL), reduced levels of high density lipoprotein (HDL) and normal or slightly elevated levels of low density lipoprotein (LDL) [9]. Taurine, 2-aminoethane sulfonic acid is a sulfur containing amino acid that is widely distributed in various animal tissues [10, 11]. It was shown to have hypoglycaemic activity, antioxidant property by reducing lipid peroxidation

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[12,-14]. and beneficial effect on blood levels of TGL and HDL in animal models [9,15]. Glycaemic control such as intensive glycaemic control can prevent micro vascular and macrovascular complications in both types 1 and 2 diabetes [16]. Glycaemic control is as such beneficial in preventing these complications; however, there is a little evidence that it promotes the average health of the diabetes. Glycaemic control may not always normalise the status of antioxidant and dyslipidaemia [17,18]. Therefore, it is paramount importance to identify suitable agents to address these concerns in diabetes. Hence, the present study was designed to investigate the effect of taurine on glycaemic control, antioxidant status and dyslipidaemia in streptozotocin (STZ) induced diabetic rats as model.

Materials and Methods

Wistar strain male albino rats of 19±1 weeks of age weighing 200-220 grams were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. They were housed in polypropylene cages; three per cage and kept at 25±2°C with 12:12 hour light and dark cycles. The animals were maintained on standard chow diet and water ad libitum. They were randomly divided into four groups and each group consisted of eight animals. Group I (control) standard chow diet; Group II (chow diet plus taurine) Group III (diabetes induced), Group IV (diabetic receiving taurine).

Induction of diabetes

The animals were fasted for 24 hour before inducing diabetes with STZ. They were injected intraperitoneally with STZ at a dose of 50 mg/kg body weight in 0.1 M citrate buffer (pH 4.5). The control animals received citrate buffer alone. Diabetes was confirmed by measuring the fasting plasma glucose concentration 48 hour after STZ injection. Animals with plasma glucose concentration above 240 mg/dl were considered to be diabetic and they were taken up for the study. Taurine was administered orally once daily at a dose of 100 mg/kg body weight.

Sample collection

At the end of 45th day, all animals were sacrificed by cervical decapitation after overnight fasting. Blood samples were collected in plain and heparinized tubes. They were centrifuged at 3000 rpm at room temperature. The serum and plasma were used for analyses. The liver specimens were collected in homogenising buffer (0.1 M Tris-Hcl, pH 7.4) for homogenisation and in 10% formalin for histopathological studies.

Biochemical analyses

Plasma glucose was determined by enzymatic glucose oxidase peroxidase (GOD-POD) method, serum total cholesterol, TGL, HDL were estimated by using Boehringer Mannheim reagent kits in Erba Smart Lab analyzer, USA. LDL was calculated using the formula developed by Friedewald et al [19]. Lecithin cholesterol acyl transferase (LCAT) activity was estimated by the method of Legraud et al [20]. with the modification of Hitz et al. (1983) [21]. Lipoprotein lipase (LPL) activity was assayed by the method of Schrecker and Greter (1979) [22]. A portion of the liver was homogenised in homogenising buffer and the homogenate was used for the estimation of hepatic fats and assay of fat metabolizing enzyme as well as determination of thiobarbituric acid reactive substances (TBARS). Hepatic lipase activity was assayed by the method of krauss et al. (l973) [23]. TBARS in plasma and liver homogenate were estimated by the methods of Yagi (1987) [24] and Ohkawa et al. (1979) [25]. Histopathological examination of formalin fixed liver tissues was performed with Hematoxylin and Eosin stain for fatty changes.

Statistical Analysis

One way analysis of variance (ANOVA) test was applied in order to evaluate any significant difference in the mean values. All values used in analysis represent the mean±SD of eight rats in each group. The results were considered statistically significant if the *p* values were 0.05 or less.

Results

Table l shows food intake, body weight changes and plasma glucose levels in experimental animals. Body weight was significantly reduced in diabetic rats while minimally decreased in diabetic rats treated with taurine although there was no significant change in plasma glucose levels in the same group (Group IV). Table 2 shows plasma TBARS, hepatic TBARS, cholesterol and TGL in experimental animals. Plasma and hepatic TBARS levels were significantly reduced in diabetic rats treated with taurine. There was a significant decrease in hepatic cholesterol and TGL in diabetic rats treated with taurine (Group IV). Table 3 shows

serum lipid profile and lipoprotein metabolizing enzymes in experimental animals. There was a significant decrease in serum cholesterol, TGL and LDL in diabetic rats administered with taurine (Group IV). The activities of LPL and LCAT were increased in Group IV compared to Group III.

Figure 1 shows histopathological changes of liver. Hepatocytes were normal with portal triad and central vein in control rats (Group I). Hepatocytes were normal with mild congestion of central vein and sinusoidal dilatation with congestion in control rats treated with taurine (Group II). Hepatocytes showed

Table 1: Food intake, body weight and plasma glucose levels in experimental animals

Groups	Food Intake (gm)	Body weight (gm)	Plasma glucose (mg/dl)
Control	18.22±0.05	244.1 ± 8.94	103.75 ± 4.77
Control+Taurine	18.35 ±0.06*	241.5 ±l0.86*!	100.25 ±2.92*
Diabetic	20.82±0.14**	202.75±13.35**!	344.13 ±41.71**#
Diabetic+Taurine	19.53 ±0.16***	221.00± 11.01***#	329.5 2±27.4***

Values are expressed as mean ± SD ; * Group 2 compared with Group 1 ** Group 3 compared with Group 1; *** Group 4 compared with Group 3 !p<0.05; #p<0.001

Table 2: Plasma TBARS, hepatic TBARS, cholesterol and TGL in experimental animals

Groups	Plasma TBARS nmoles/ml	Hepatic TBARS nmoles/mg pro	Hepatic Cholesterol mg/l00gm	Hepatic TGL mg/100gm
Control	0.37±0.04	0.34±0.40	318.0± 5.86	335.5 ± 6.23
Control + Taurine	0.29±0.03*#	0.28±0.42*	310.0±6.23*	326.4 ±5.42*
Diabetic	0.61±0.04**#	0.49±0.06**#	534.13±15.15**#	480.4 ±9.23**#
Diabetic+Taurine	0.43±0.03***#	0.37±0.02***#	322.62±8.94***#	372.3± 4.34***!

Values are expressed as mean ± SD; *Group 2 compared with Group 1; ** Group 3 compared with Group 1 ***Group 4 compared with Group 3; !p<0.05 #p<0.001

Table 3: Serum lipid profile and lipoprotein metabolising enzymes in experimental animals

Group	Cholesterol mg/dl	TGL mg/dl	LDL mg/dl	LPL	LCAT	HDL mg/dl
Control	87.13±8.54	95.5±7.17	35.15±9.96	0.13±0.04	0.67±0.04	32.13±2.42
Control+Taurine	90.88±10.55*	93.25±16.32*	36.6±7.70	0.14±0.03*#	0.70±0.06*	35.38± 2.78*!
Diabetic	228.0±15.72**#	258.63±22.66**#	150.40±13.37	0.05±0.02**#	0.23±0.02**#	26.13± 3.19**#
Diabetic+Taurine	140.5±29.95***#	168.25±11.17***#	76.22±31.33	0.113±0.05*** #	0.58±0.02***#	30.38±3.02***!

Values are expressed as mean ± SD; *Group 2 compared with Group 1; ** Group 3 compared with Group 1 ***Group 4 compared with Group 3; !p<0.05; #p<0.001
LPL: µ moles of glycerol liberated/ml/hr;
LCAT: n moles of cholesterol esterified /ml/hr

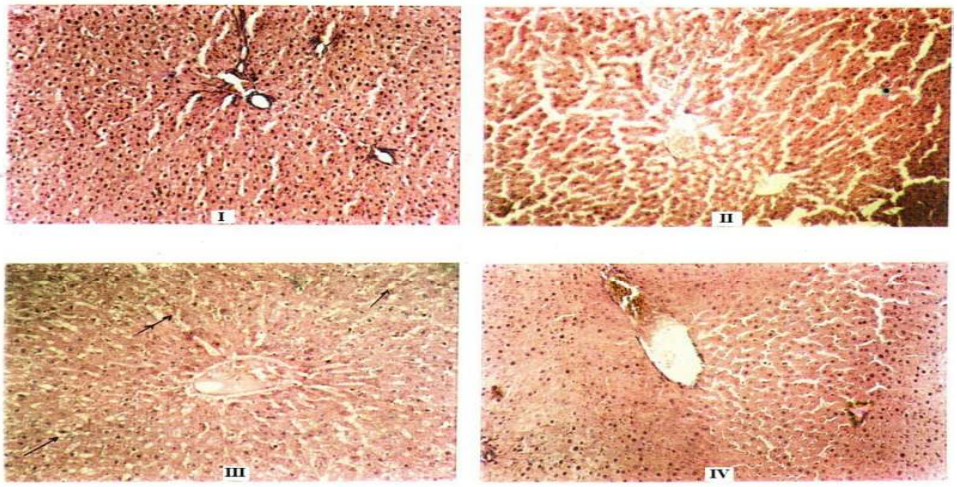

Fig. 1: Histopathology of liver H&E x 100

sinusoidal dilatation and congestion of central vein with micro vascular type of fatty changes in diabetic rats as indicated with black arrows (Group III). Hepatocytes showed congestion of central vein and focal sinusoidal dilatation with no fatty changes in diabetic rats treated with taurine (Group IV).

Discussion

The present study investigated the effect of taurine on hyperglycaemia, antioxidant and dyslipidaemia in STZ induced diabetic rats. It was found that taurine did not improve the glycemic status as taurine administration showed no significant effect on plasma glucose levels in taurine treated group. This is in contrast to earlier studies which reported that taurine was effective in reducing hyperglycaemia [26,27]. Taurine administration reduced the plasma and hepatic TBARS levels significantly which suggests the antioxidant role of taurine which is in good agreement with most other studies [14,28]. It is reported that that taurine plays a role as antioxidant by increasing the activity of super oxide dismutase (SOD) in serum and the expression of heme oxygenase-1 in liver tissue [29]. There was a significant increase in total cholesterol, LDL and TGL and decrease of HDL in diabetic rats when compared with the controls. Taurine administration reduced the blood cholesterol levels in STZ induced diabetic rats. This could be due to decreased dietary intake or decreased absorption of cholesterol. It could also be due to decreased synthesis or increased elimination or both. Decreased dietary intake could not be the cause as evident from table 1. There was no significant change in blood and hepatic lipids in control rats receiving taurine compared to control rats. Hence decreased absorption of cholesterol and decreased synthesis could be ruled out. Therefore, it might be due to enhanced elimination of cholesterol. It has been reported that taurine enhances 7-alpha hydroxylase, a key enzyme in bile acid synthesis [30,31] and hence enhanced elimination of bile acids.

Taurine administration reduced the serum and hepatic TGL levels significantly; it could not be due to decreased synthesis of TGL as taurine has no effect on insulin secretion, which is evident by insignificant change in plasma glucose levels. It suggests that taurine might reduce the lipolysis in the adipose tissue, causing lowering of serum and hepatic TGL and so only marginal weight loss was seen when compared to diabetic rats. This might be due to enhancement of LPL activity and increased utilization of TGL rich lipoproteins by peripheral tissues or due to decreased uptake by liver. Taurine administration enhanced the LCAT activity which shows that there is an increased uptake of cholesterol. But the hepatic cholesterol was significantly reduced which implies that cholesterol might be converted to bile acids which would be easily eliminated [32-34]. There was a significant reduction of hepatic fats which suggests the protective role of taurine against fatty changes due to diabetes in liver (Figure 1).

Conclusion

The present study indicates that taurine supplementation might be beneficial in alleviating oxidative stress and dyslipidaemia in diabetes.

Conflict of Interest: None

Key Messages

Diabetes mellitus is characterised by chronic hyperglycaemia. It is also characterised by lipid peroxidation and dyslipidemia. It is associated with complications such as micro and macro vascular complications. Taurine supplementation would be beneficial to these complications in diabetes.

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Preparation and Organoleptic Characteristics of Flaxseed Supplemented Products

Priyanka Kajla[1], Alka Sharma[2]

Abstract

In the present investigation three different products viz., idli, porridge and chutney were prepared using standardized recipes. The standard products were supplemented with various levels of flaxseeds. The aim of the study was to select the best supplementation level of flaxseed in these products. It was found that 10% supplementation level of flaxseeds was found to best in terms of sensory characteristics.

Keywords: Porridge; Flaxseeds; Chutney; Idli; Supplementation.

Introduction

Flaxseed is now-a-days emerging as a potential food crop as functional food. Amazingly good source of quality nutrients –omega-3 fatty acids, quality protein, lignans, soluble and insoluble dietary fibre make it a wonder functional food source. Alarming increase in obesity and various chronic diseases, people are shiftings towards the inclusion of flaxseed in the routine as functional foods and nutraceuticals [1,2]. In recent years, as people have become more aware and conscious about health issues, therefore the demand for flaxseeds in food and beverages, functional foods and dietary supplements has risen drastically both in the United States and other developing countries. Flaxseeds can be utilized in form of roasted, germinated and milled seeds, while flaxseed oil can be used in various food formulations as salad dressings, as an ingredient in ice-cream as stabilizer and also as micro- and nano-encapsulated powder. Bakery sector so far has best utilized the flaxseed in various bakery products formulations to meet customer demands. Flax or flaxseed oil has been incorporated into baked foods [3], fruit beverages, milk and other dairy products [4], muffins [5], dry pasta products [6], macaroni [7]. FDA has authorized up to 12% incorporation of flaxseeds in foods, and this may be taken as a representative maximum addition level for most of the foods [8].

In India, a variety of chutneys and pickles are prepared using different vegetables, pulses and spices that are consumed with breakfast items like chapatti, idli, dosa. Literature is available on development and standardization of several food adjuncts namely traditional chutneys instant chutneys and chutney powders, instant multigrain porridge, based on the various raw materials available during different seasons [9]. Interestingly, there was no literature available on utilization of flaxseed in products like porridge, idli and chutney, therefore the present investigation was planned to prepare these products and also quality evaluation of these products were also done to check the effect of processing on the nutritional quality of the prepared products.

Materials & Methods

Roasted and Germinated flaxseed powders were selected on the basis of studied nutritional [10] and organoleptic characteristics for prepared products. Three different types of products were prepared by supplementation- Porridge, Idli and Chutney.

Flaxseed Supplemented Idli

For the preparation of idli, roasted semolina

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was mixed all other ingredients and was blended together. Proportion of flaxseed was varied from 5g to 20 g in the above blend. The blend was allowed to stand for 10 minutes. Then the whole mixture was steamed for 15 minutes.

Table 1: Various trials of flaxseed supplemented chutney

Flaxseed supplementation level	0(%)	5	10	15	20
Ingredients (g)					
Semolina	25	25	25	25	25
Flaxseeds	0	5	10	15	20
Curd	5	5	7	10	10
Leavening agent	0.25	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50	0.50

Flaxseed Supplemented Porridge

Porridge was prepared using the traditional recipe by boiling the coarsely ground wheat (100g) in 350 ml water for 30 minutes in covered pan. The level of supplementation of flaxseed varied from proportion of wheat: porridge -100:0; 95:5; 90:10; 85:15; 80:20. All the trials were evaluated for various sensory characteristics and best supplementation level was selected on the basis of maximum sensory scores.

Flaxseed supplemented Chutney

50 parts of roasted and germinated flaxseed flour was mixed with other ingredients namely green chillies (10 parts), coconut (5 parts), mint leaves (5.5 parts), garlic (5.5 parts), tamarind (7 parts), jaggery (4 parts) and along with common salt and dry mango powder (13 parts) blended in a laboratory mixer to obtain flaxseed chutney. The recipe for the preparation of flaxseed supplemented chutney was standardized in several trials by varying the quantities of roasted and germinated flaxseed powder and other ingredients in different proportions -25:75, 30:70, 50:50, 60:40 and 80:20.

Table 2: Various trials of flaxseed supplemented chutney

Supplementation level (%)	25:75	30:70	50:50	60:40	80:20
Ingredients (g)					
Flaxseeds	25.0	30.0	50.0	60.0	80.0
Green chillies	15.0	14.0	10.0	8.0	4.0
Coconut	7.5	7.0	5.0	4.0	2.0
Garlic	8.25	7.7	5.5	4.4	2.2
Mint	8.25	7.7	5.5	4.4	2.2
Tamarind	10.5	9.8	7.0	5.6	2.8
Jaggery	6.0	5.6	4.0	3.2	1.6
Salt and Dry mango powder	19.5	18.2	10.4	10.4	5.2

Sensory evaluation

The supplemented recipes were subjected to sensory evaluation using 9 point hedonic rating scale such as appearance, odour, taste and flavour to find the overall acceptability. The recipes were evaluated by a panel of semi trained panellists. Sensory evaluation of standard recipes on 0 (disliked extremely)-9 point (liked extremely) scale.

Results & Discussion

Preparation of flaxseed supplemented idli and its sensory evaluation

Supplemented idli was selected for further studies on basis of sensory characteristics. Data pertaining to the various levels of supplemented idli is presented in Table 3.

Scores for various sensory parameters presented in table clearly depicted that idli prepared with incorporation of 10% flaxseed was found to be best in terms of good overall acceptability in comparison to other supplementation levels. 10% flaxseed supplemented idli scored maximum for colour (6.19), texture (5.85), aroma (6.67), appearance (6.80) and taste (7.08). While control idli i.e. without addition of flaxseeds scored maximum value for all sensory characteristics as compared to various levels of flaxseed supplemented idli. As the level of supplementation of flaxseeds increased in idli sensory scores followed a significant decreasing trend i.e. idli prepared by the incorporation of 20% flaxseeds showed poor sensory scores for all sensory parameters. Therefore, 10% supplementation level was selected for further studies. Then, the idli was prepared for further studies by incorporating 10% raw, roasted and germinated flaxseeds.

Preparation of flaxseed supplemented porridge and its sensory evaluation

Porridge was supplemented with the flaxseeds @ 5% to 20% and then sensory evaluation was done to found out best acceptable level of acceptability of flaxseed in porridge.

On the basis of sensory scores, best acceptable supplementation level was selected for further studies.

Data pertaining to sensory characteristics of flaxseed supplemented porridge is presented in Table 4. The flaxseed porridge was evaluated for various sensory parameters such as colour, texture, aroma, appearance and taste etc. In the wheat porridge flaxseeds were incorporated @ 5%

to 20%. On the basis of overall acceptability best supplementation level was selected. As the data related to overall acceptability shown in Table 4 revealed that wheat porridge supplemented with 10% flaxseeds scored highest for all sensory parameters. The score of overall acceptability was 7.76 for 10% supplemented porridge followed by & 7.52 for 5% supplementation porridge. The scores for sensory parameters for control/ wheat porridge were highest among all types of supplemented porridge. On the hand among the flaxseed supplemented porridge, 20% flaxseed supplemented porridge scored lowest values for sensory characteristics. Therefore, on the basis of 10% flaxseed supplemented porridge was selected for further quality analysis.

Preparation of flaxseed supplemented chutney and its sensory evaluation

Various trials of flaxseed supplemented were prepared in different proportions and then sensory evaluation was done to found out best acceptable level of acceptability of flaxseed in chutney. On the basis of sensory scores, best acceptable supplementation level was selected for further studies

Data pertaining to sensory characteristics of flaxseed supplemented chutney is presented in Table 5. The flaxseed chutney was evaluated for various sensory parameters such as colour, texture, aroma, appearance and taste etc. In the chutney flaxseeds were incorporated 25g-80g in proportion to other ingredients. On the basis of overall acceptability best supplementation level was selected. As the data related to overall acceptability shown in Table 5 revealed that control chutney supplemented with 50:50 flaxseeds: ingredients scored highest for all sensory parameters. The score of overall acceptability was 6.54% for 50:50 supplemented chutney followed by & 6.14% for 30:70 supplementation chutney. The scores for sensory parameters for control chutney were highest among all types of supplemented chutney. On the hand among the flaxseed supplemented chutney, :8020 flaxseed supplemented chutney scored lowest values for sensory characteristics. Therefore, on the basis of 50:50 flaxseeds supplemented chutney was selected for further quality analysis.

Table 3: Sensory characteristics of flaxseed supplemented Idli

Flaxseed supplementation	Colour	Texture	Aroma	Appearance	Taste	OA
0(%)	7.16±0.40e	6.84±0.04e	6.83±0.44e	7.22±0.07e	7.63±0.54e	7.12±0.29e
5(%)	6.67±0.51d	5.42±0.05c	6.33±0.54c	6.41±0.04c	6.42±0.43c	6.25±0.32c
10(%)	6.19±0.40c	5.85±16 .0d	6.67±0.47d	6.80±0.44d	7.08±0.08d	6.51±0.44d
15(%)	5.34±0.56b	5.41±48 .0b	5.16±0.03b	5.41±0.57b	5.43±0.23b	5.35±0.09b
20(%)	3.83±0.75a	4.63±0.08a	4.17±0.07a	4.23±0.09a	4.41±0.77a	4.25±0.26a

Values are means of 8 values± SD. Means in each column with different letters are significantly different. OA-Overall acceptability

Table 4: Sensory characteristics of flaxseed supplemented porridge

Wheat:Flaxseed (g)	Colour	Texture	Aroma	Appearance	Taste	OA
100:0	8.140.40±e	8.860.04±e	8.790.44±e	8.490.07±e	8.630.54±e	8.580.29±e
95:5	7.870.51±d	7.440.05±c	7.290.54±c	7.560.04±c	7.450.43±c	7.520.32±c
90:10	7.890.40±c	7.8516 .0±d	7.670.47±d	7.600.44±d	7.800.08±d	7.76±0.44d
85:15	6.140.56±b	6.1148 .0±b	6.160.03±b	6.410.57±b	6.430.23±b	6.250.09±b
80:20	5.830.75±a	4.220.08±a	4.290.07±a	4.360.09±a	4.410.77±a	4.620.26±a

Values are means of 8 values± SD. Means in each column with different letters are significantly different. OA-Overall acceptability

Table 5: Sensory characteristics of flaxseed supplemented chutney

Flaxseed:Ingredients	Colour	Texture	Aroma	Appearance	Taste	OA
25:75	6.67±0.65d	7.36±0.49e	7.02±0.06d	7.41±0.23e	7.81±0.27e	7.25±0.38e
30:70	6.34±0.06c	6.33±0.17c	5.21±0.08b	6.24±0.58c	6.61±0.53c	6.14±0.48c
50:50	6.51±0.38c	6.85±0.42d	5.63±0.12c	6.65±0.57d	7.07±0.28d	6.54±0.49d
60:40	5.00±0.52b	4.84±0.11b	5.12±0.45b	5.41±0.11b	4.65±0.39b	4.98±0.25b
80:20	3.82±0.11a	3.83±0.32a	4.43±0.13a	3.86±0.42a	3.69±0.71a	3.92±0.25a

Values are mean of 8 values±SD. Means with different letter are significantly different

Conclusion

From the present study it can be concluded flaxseeds can be supplemented up to 10% level. This supplementation level is best acceptable level organoleptically as well as nutritionally.

Incorporation of flaxseeds into the traditional products like idli, porridge not only improved the nutritional profile but also its nutritional quality is maintained while processing by decreasing antinutritional factors to a greater extent. Therefore flaxseed can be incorporated into various reciepes to a wide variety of value added nutritional product at economical rates.

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A Study on Food Fortification to Alleviate Anaemia

E. Lakshmi

Abstract

Context: The juice of raw beetroot contains host of health benefits and is classed as a "super food" in today's nutritional jargon for anaemia. *Aims:* To determine the effectiveness of enriched beetroot juice on serum iron level and clinical symptoms between study and control group of adolescent girls. *Settings and Design:* The sample size selected for this study consists of 80 adolescent girls of age 13-18 years (Haemoglobin <9gm/dl) assigned to study and control group. *Methods and Material:* 150ml of enriched beetroot juice was served to each of the study subject every alternate days for three months (45 days). Control group was served with a placebo. Nutrition education was given to all the participants(n=80). serum iron (dipyridyl method) was assessed and nutritional assessment for iron deficiency anaemia was performed. *Statistical analysis used:* Descriptive statistics like percentage and paired 't' test was performed. *Results:* 50 per cent of subjects suffering from fatigue and feeling of weakness (13-15yrs) reduced to 10 per cent after fortified beet-root extract intervention. The mean serum iron (16-18yrs) level improved significantly (t=13.86,p< .0001) in the study group. *Conclusions:* Enriched beet-root juice supplementation improved the nutritional status and serum iron levels of adolescents.

Keywords: Enriched Beet-Root Juice; Anaemia; Supplementation; Serum Iron; Placebo.

Introduction

Adolescents are particularly vulnerable to nutrient deficiencies especially iron leading to anaemia. Without added vitamins and minerals, many children and teens don't meet daily nutrient requirements [1]. Fortified and enriched foods are important sources of nutrients for the growing period, especially for iron, zinc, and B vitamins they can fill in the gaps and increase a particular vitamin and mineral consumption that would otherwise be less than the recommended value [2].

Though iron and folic acid supplementation remains the corner stone in treatment of anaemia nutrition education and food supplementation are long term measures in preventing the recurrence.

A food-based strategy has the goal of improving nutrition through increasing the availability and consumption of a nutritionally adequate micronutrient rich diet made up of a variety of available foods [3].

Good nutrition is not just about having enough food, but about having the right food, at the right times. The food we eat must contain enough nutrients otherwise we can become malnourished.

Food fortification is one important step in making sure populations gain a balanced diet. It helps to fill micronutrient gaps where populations struggle to access nutritious foods, which is especially important as populations grow, live in more urban areas, and consume more processed foods rather than freshly grown food [4].

A number of potential dietary sources need to be urgently promoted including many leafy vegetables like beet-root The process of selecting the best food vehicle and iron source may appear simple but is actually a complex process that requires evaluation at every step [5]. Beet-root juice is possibly the best natural remedy for anaemia. It helps to increas the blood count and improve blood circulation and oxygen carrying capacity of erythrocytes [6].

The study was conducted to assess the prevalence of anaemia among adolescent (12-18yrs) girls and estimate the impact of supplementation of iron rich beet-root juice on serum iron values.

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Subjects and Methods

Government- aided girls school of Chennai city was selected and designed to include eligible adolescent students in the age group of 12 – 18 years. Ethical approval was obtained from school authorities, students and parents. They were briefed about the supplementation study with fortified beetroot juice.

Baseline haemoglobin (cyanmethaemoglobin) was assessed for 118 adolescent students (12–18 yrs). 98 adolescents were found to be anaemic (Haemoglobin <9gm/dl). 80 study participants were randomly assigned to two groups. The study group and control group. The participants were appraised of anaemia and the importance of fortified beetroot extract in combating anaemia through nutrition lecture.

The concept of the enriched product took shape after a series of trials using ingredients in various proportion. Enriched beet-root juice was processed by grinding 1 kilogram of beet root with 10gms ginger, 500 grams sugar the juice extracted with 2 litres of water and 30ml of fresh-lime juice. 150ml of beetroot juice was given to each of the intervention subject on every alternate days for three months (45days).

Serum iron (dipyridyl method) was estimated at pre and post test. The study participants were screened for clinical symptoms pertaining to nutritional iron deficiency anaemia based on standard WHO (1963) procedure.

Students paired 't' test was performed to find the difference between pre and post test.

Results

Distribution of subjects according to clinical signs and symptoms of anaemia (Table 1) showed that 55 per cent of the subjects depicted pale skin followed by 90 per cent with pigmentation of nails. Drinking beet-root juice helps in healthy glowing skin [7]. Impact of enriched beet-root juice supplementation reduced the symptoms. Whereas 50 per cent of subjects suffering from fatigue and feeling of weakness reduced to 10 per cent after fortified beet-root juice intervention, while Poor appetite was reported by 30 per cent of adolescent subjects. Frequent headache was reported by 50 per cent of subjects. After fortified beet-root juice intervention the symptoms reduced to 10 per cent. Beetroot juice is particularly beneficial as an anemia remedy for children and teenagers. consuming beet root juice or beet as cooked vegetable in salad is highly beneficial in treating anemia [8].

Food-based intervention programs like dietary enhancement and diversification, food fortification including bio fortification play a critical role in alleviating micronutrient deficiencies [9]. In the Post-adolescent (16-18yrs) age group as evident from Table 2 about 75 (n=15) per cent of subjects depicted skin paleness in the pre intervention group, pigmentation of nails were observed in 40 per cent. It was observed that 75 per cent (n=15) of subjects were reported to suffer from fatigue and feeling of weakness The impact of enriched beet-root juice supplementation reduced the symptoms to 10 per cent.

The detoxifying ability of beet-root helps in curing skin problem and helps to hydrate the skin. The impact of nutrition education alone in the control group not much impact was observed at the post test stage. Adolescence is a period of rapid growth with stress and strain. Frequent headache reported by 80 per cent of subjects in the pre intervention group reduced to 5 per cent.

Serum iron test measures the amount of iron in the blood A significant increase ($t=16.97$, $p<.0001$) in the mean serum iron level (Table 3) was observed (13-15years). The baseline value 55.30±2.37 gradually increased to 109.40±9.49 after three months of intervention. In the control group the increase was not significant ($t=0.26$, $p=0.79$). Fortified beet-root extract intervention for three months had high significance ($t=15.57$, $p< .0001$) in the mean serum iron level compared to control.

The antioxidant property of beetroot helps to scavenge free radicals to prevent aging, cancer and reduce blood pressure to help the muscles in uptake of oxygen [10]. There was significant ($t=13.86$,$p< .0001$) increase in the mean serum iron level (16-18yrs) of the post- intervention group. The baseline (54.85±3.0) serum iron gradually increased (104.75±6.71) after three months of Supplementation. Whereas the mean difference (1.7±1.27) in the control group was not significant ($t=2.78$, $p=0.011$). Beeturia is a term applied to the deep red or pink coloration of urine which may occur after the ingestion of beetroot [11]. The impact of fortified beet-root extract intervention in the mean serum iron level for three months was statistically significant ($t=14$, $p< .0001$) compared to control.

Table 1: Nutritional assessment of adolescent girls

S.No	Factors	13-15years							
		Study (n=20)				Control (n=20)			
		Pre-test		Post-test		Pre-test		Post-test	
		No	Percent	No	Percent	No	Percent	No	Percent
1	Pallor of skin	11	55	1	5	10	50	10	50
2	Pigmentation of nails	18	90	-	-	16	80	11	55
3	Fatigue and weakness	10	50	2	10	10	50	8	40
4	Bleeding gums	3	15	-	-	2	10	-	-
5	Tooth decay	5	25	1	5	4	20	2	-10
6	Frequent headache	10	50	2	10	13	65	11	55
7	Poor appetite	6	30	2	10	4	20	4	20

Table 2: Nutritional assessment of adolescent girls

S. No	Factors	16-18years							
		Study (n=20)				Control (n=20)			
		Pre-test		Post-test		Pre-test		Post-test	
		No	Percent	No	Percent	No	Percent	No	Percent
1	Pallor of skin	15	75	2	10	13	65	9	45
2	Pigmentation of nails	8	40	-	-	7	35	6	30
3	Fatigue and weakness	15	75	-	-	16	80	13	65
4	Bleeding gums	2	10	-	-	2	10	-	-
5	Tooth decay	4	20	-	-	5	25	3	15
6	Frequent headache	16	80	1	5	13	65	10	50
7	Poor appetite	10	50	2	10	10	50	12	60

Table 3: Mean serum iron values of adolescents

Groups	Baseline	Final 3rdmonth 45th day	Mean difference	T value		Sood 1990
Experimental (13-15yrs) A_1	55.30±2.37	109.4±9.49	54.1±9.11	16.97	p <.0001	60-160µg/dl
Control (13-15yrs) B_1	54.2± 2.89	54±2.9	0.20 ±1.63	0.26 A_1vs$B_1$15.57	P= 0.7976 p <.0001	
Experimental (16-8yrs) A_2	54.85±3.0	104.75±6.71	49.90±7.52	13.86	p <.0001	
Control (16-18yrs) B_2	51.15± 4.35	52.85 ±4.33	1.70±1.27	2.78 A_2vs$B_2$14	P= 0.0119 p <.0001	

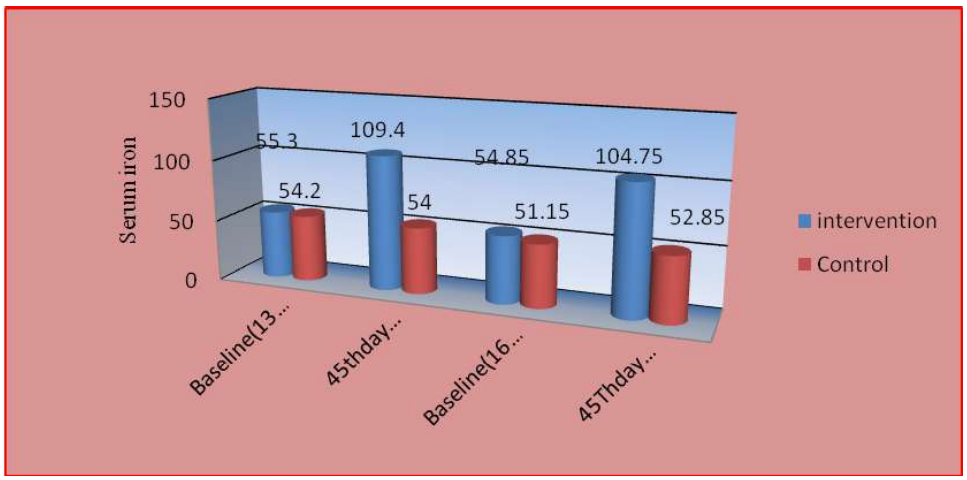

Fig. 1: Mean serum iron values

Discussion

Serum iron status improved with fortified beet-root juice supplementation for three months (45days) and clinical symptoms related to nutritional iron deficiency anaemia improved significantly. Fatigue and weakness completely reduced. The baseline 55.3±2.37 serum iron value (13-15years) gradually increased to 109.4±9.49 after three months of supplementation. Fortified beet-root juice supplementation for three months had high significance ($t=15.57$, $p< .0001$) in the mean serum iron level compared to control. The baseline (54.85±3.0) serum iron (16-18yrs) gradually increased (104.75±6.71) after three months of intervention.

Fortified beet-root juice supplementation on alternate days along with nutrition education helped to improve the nutritional status and serum iron index. The study revealed that organic forms of nutrients derived from natural sources are much easier to assimilate than synthetic nutrients. Thus the iron content in beetroot juice is easily assimilated and helps to increase the serum iron index.

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Key Messages

The beetroot juice contributes to improve the nutritional status and serum iron levels of adolescents. The cost of the beetroot is low when compared to other iron rich vegetables and it can be stored easily. Hence the present study was conducted on adolescent girls.

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Types of Manuscripts and Limits

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

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I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

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If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, 45796900, 22756995. E-mail: author@rfppl.co.in. Submission page: http://rfppl.co.in/article_submission_system.php?mid=5.

Preparation of the Manuscript

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Figures, Figure legends, and Acknowledgment. Do not make subheadings in these sections.

Title Page

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)
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l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

Abstract Page

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

[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.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.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.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc).

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Tables with more than 10 columns and 25 rows are not acceptable.

Table numbers should be in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

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Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

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Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

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