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A Comparative Observational Study of Postmortem Computed Tomography and Traditional Forensic Autopsy Findings in Hanging Cases

Anam Khan[1], Karthi Vignesh Raj K[2], Abhishek Yadav[3], Sudhir K. Gupta[4], Varun Chandran A[5], Abilash S[6], Swati Tyagi[7]

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Abstract

Background: The use of advanced radiological techniques is rising in the field of forensics to supplement and validate the evidence. Postmortem Computed Tomography (PMCT) is now used as a standard procedure in many institutions to augment traditional autopsy findings. The application of PMCT in hanging cases will greatly reduce the number of invasive traditional autopsies, as hanging is the most common method of suicide in Northern parts of India. Therefore this observational study was conducted to compare PMCT with traditional autopsy in fifty hanging cases.

Result: Out of 50 cases, the age of the subjects ranged between 14 years and 70 years. PMCT with the help of 3D reconstruction was able to detect external ligature marks on the neck in 92% (N=46) of cases giving a sensitivity of 92% (CI = 80.77% to 97.78%). Skin and subcutaneous tissue desiccation were identifiable in 96% (N=48) by PMCT, giving a sensitivity of 96% (CI= 86.29% to 99.51%). The muscle hemorrhage was identified in 4% (N=2) of the cases in traditional autopsy while the authors found difficulty in interpreting the muscle hemorrhage in PMCT. In detecting thyroid cartilage fracture, the sensitivity of PMCT was found to be 100% (CI= 15.81% to 100.00%) and specificity of 100% (CI=92.60% to 100.00%). In detecting hyoid bone fracture, the sensitivity was 100% (CI= 2.50% to 100.00%) and specificity of 100% (CI=92.60% to 100.00%). In addition, there was a great degree of agreement between the two observers for these findings suggesting the reproducibility of the result.

Conclusions: A consistency in findings of both the PMCT and traditional autopsy was found. However, CT was unable to detect muscle hemmorhages, compared to traditional autopsy. Authors suggest the use of CT angiography to overcome this shortcoming, till then CT can only augment invasive autopsy findings not replace it.

Author Affiliation: [1]Ex-Junior Resident, [2]Senior Resident, [3]Additional Professor, [4]Professor and Head, [5]Assistant Professor, [6,7]Senior Medical Officer, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

Corresponding Author: Abhishek Yadav, Additional Professor, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

E-mail: drayad_in@yahoo.com

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INTRODUCTION

Hanging is a form of asphyxia where there is constriction of the neck and this constricting force applied to the neck comes from the gravitational drag of the weight of the body or a

part of it.[1] These asphyxia deaths though common are quite complex and need meticulous examination by an autopsy surgeon. In such a situation any tool or investigation supporting the findings will be of immense help. Internal injuries such as fractures and dislocations of hyoid and thyroid cartilage, and neck muscle hemorrhages are of significant value in forensic pathology, so as to better understand the constrictive force over the neck and to estimate the intensity of the force applied. The severity of internal neck injuries can not be estimated by solely looking at the external findings.[2] The external lesions may only be discreet or sometimes even absent, which further necessitates the requirement to apply advanced radiological techniques. Currently, for hanging cases, the traditional invasive autopsy is considered the gold standard. It is not just time consuming, but also requires a lot of precision and experience.[3] But even then the fractures of the thyroid and hyoid may be missed or misinterpreted due to artifacts produced by the dissection, therefore using additional radiological tools to augment the traditional autopsy findings is very much needed.[4]

The use of CT and MRI in the field of forensic medicine has been validated and its usefulness is established by many studies in routine medicolegal death investigations.[5-7] Implementing these diagnostic tools would greatly increase the standard and quality of autopsy, which could be reproducible evidenceto the legal system. In addition, the correlation of macroscopic, microscopic, and radiological findings will enhance the scientific validity of forensic evidence. There have been several studies in the past discussing radiological neck findings in cases of hanging and ligature strangulation. Wallace et al described the cervical spine abnormalities and subarachnoid hemorrhage in two cases of judicial hanging.[8] Hayashi et al in their study reported a case of hangman's fracture in non-judicial hanging detected by PMCT among his 32 cases of hanging.[9] Kemper et al in their study claim that the use of post-mortem Multislice Computed Tomography (MSCT) along with the thorough external examination and crime-scene investigation can further guide a need for more invasive investigation.[2] All these studies highlighted the importance of radiological investigation in hanging cases. Therefore, the authors compared the traditional autopsy and Postmortem MSCT internal neck findings in 50 hanging cases. The authors aim to validate the PMCT findings to determine the cause and manner of death in comparison with Traditional autopsy and to replace traditional invasive autopsy with the PMCT in future cases.

METHODS

Study Population

In the month of August and September, 50 hanging cases were done at the Department of Forensic Medicine and Toxicology, AIIMS, New Delhi which were taken for this study. These 50 cases had the cause of death as asphyxia due to hanging. All presented some form of ligature mark on the neck. Out of the total 50, 74%(N=37) were male and 26%(N=13) were females.

Post-mortem Imaging

In each case, an autopsy was preceded by a PMCT examination using a 16 slice MSCT spiral scanner, Canon, Aquillon Lightning TSX-035A CT. The scanning parameters used were 120kV and 70 mAs. 16 x 1 mm collimation was used for all the cases for data acquisition. All the raw data was processed into slices of 1mm thickness. Multiplanar and 3D reconstruction were also done. The processed data were assessed and reported at two dedicated workstations (Vitrea 6.9. and Vitrea 7.10). A group of forensic doctors with training in post-mortem radiological assessment interpreted the findings.

Traditional Autopsy

Following imaging, a standard traditional autopsy using the Letulles technique of dissection was conducted by forensic doctors. This method of the autopsy was chosen as it gives detailed layer wise dissection findings while retaining anatomical correlation. It has better compliance and can easily detect injuries.

Statistical Analysis

The sensitivity and specificity of each variable were calculated. In addition, to prove, the reproducibility of the data inter-observer tests were applied. For inter-observer variability cases were analyzed by another forensic doctor with prior training in radiology.

RESULTS

Descriptive Statistics

A total of 50 cases were analyzed in the study. The age of the subjects varied between 14 years and 70 years, with a median age of 44 years.

Sensitivity and Specificity of PMCT

An evaluation of the parameters (Table 1) showed that PMCT with the help of 3D reconstruction was able to detect external ligature marks on the neck in 92% (N=46) of cases giving a sensitivity of 92% (CI=80.77% to 97.78%). Skin and subcutaneous tissue desiccation were identifiable in 96%(N=48) by PMCT, giving a sensitivity of 96% (CI= 86.29% to 99.51%). The muscle hemorrhage was identified in 4%(N=2) of the cases in traditional autopsy where the authors found difficulty in interpreting the same at PMCT. In detecting thyroid cartilage fracture, the sensitivity of CT was found to be 100% (CI=15.81% to 100.00%) and specificity of 100% (CI=92.60% to 100.00%). In detecting hyoid bone fracture, the sensitivity was 100%(CI=2.50% to 100.00%) and specificity of 100% (CI=92.60% to 100.00%). Out of the total 50 cases, 26% (N=13) had ligature material in-situ. Among the ligature material, the most commonly encountered was cotton cloth in the form of a bedsheet or dupatta (shawl) in 10 cases and the remaining 3 cases had a rope as presented in Table 2.

Table 1: Comparison of various parameters in Traditional Autopsy and PMCT

Parameter	Autopsy Findings present in	PMCT Findings present in
External neck ligature mark	100%(N=50)	92%(N=46)
Skin and Subcutaneous tissue desiccation	100%(N=50)	96%(N=48)
Intramuscular hemorrhage	4%(N=2)	0
Fracture of the Hyoid bone	2%(N=1)	2%(N=1)
Fracture of Thyroid cartilage	2%(N=1)	4%(N=2)

Table 2: Ligature Material In-situ

Case No.	Age	Gender	Ligature Material	Hounsfield Units(HU) on CT
1	70 years	Male	Nylon Rope	650-900
10	24 years	Male	Bedsheet	600-850
11	22 years	Male	Dupatta	500-700
13	60 years	Male	Nylon Rope	500-800
16	20 years	Female	Soft cloth	600-700
18	25 years	Male	Bedsheet	600-750
24	17 years	Male	Bedsheet	750-850
32	59 years	Male	Nylon Rope	600-700
34	21 years	Male	Bedsheet	800-1000
39	29 years	Female	Bedsheet	800-1000
44	52 years	Male	Bedsheet	600-700
47	44 years	Male	Cotton Cloth	600-700
50	44 years	Male	Cotton Cloth	500-650

INTEROBSERVER ANALYSIS

An interobserver agreement analysis was performed to calculate the intraclass correlation coefficient (ICC) between the first and the second observer for various variables observed. (Table 3)

Table 3: Interobservor analysis

Variable	Interobserver Cohen's kappa	Agreement %
External ligature mark	0.634	94%
Skin and Subcutaneous Tissue	0.789	97%
Thyroid fracture	0.657	98%
Hyoid Fracture	1	100%

DISCUSSION

The external ligature mark is mostly visible well on external examination, but if it could be recorded in Post-mortem CT thenit could be a reproducible, reliable, and decision making toolat later stages also as it decreases the need for exhumationin disputed cases. The external ligature mark on 3 D reconstruction was appreciated in 92% of all the cases (fig. 1). The presence of a faint external mark on the bodyin the remaining cases resulted ina difficult detection on CT by the authors. This was observed in cases where the ligature material used was a soft cotton material likea bedsheet, which was also reported in previous studies.[10]

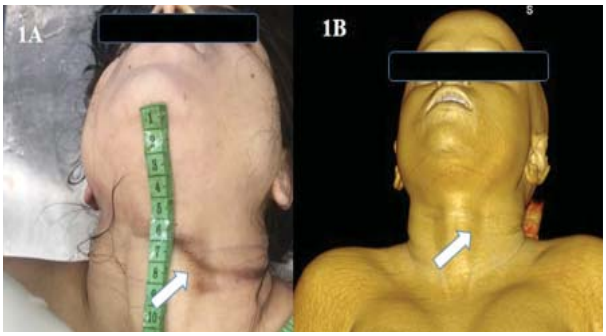


Fig. 1a: External ligature mark on Traditional autopsy.

Fig. 1b: External ligature mark on 3D reconstructed CT image.

The primary signs of hanging like skin changes and subcutaneous desiccation were found in almost all of the hanging cases in both the traditional autopsy and the PMCT. It is in concordance with the finding of Yen et al. who reported that 100% of cases have these signs.[11] M Pollacco et al found the presence of ligature marks similar in both the traditional autopsy and that in PMCT.[12]

Detecting soft tissue or muscle hemorrhages in CT is difficult. According to D. Gascho et al., PMCT has missed several hemorrhages in contrast to their presence in autopsy.[13] G. Graziani et al. concluded that one of the most missed findings in CT neck was the soft tissue hemorrhage, the second being the thyroid nodules and superficial incisions.[14] In our study, muscle hemorrhage was found in 2 of the 50 cases in traditional neck dissection, but none was interpreted by the authors in CT (fig. 2). MSCT-based post-mortem angiography can be used to not only detect vascular injuries but also to merit the contrast agent leakage to detect hematomas, which are missed on native PMCT.[15] However, the authors coundnt find any findings or technique supportive of detecting very small hemorrhages using contrast agents, which could be a significant finding in hanging cases. The absence of detection of the hemorrhage on CT makes it difficult to call any fracture antemortem or post-mortem, as the presence of hematoma validates its antemortem nature.

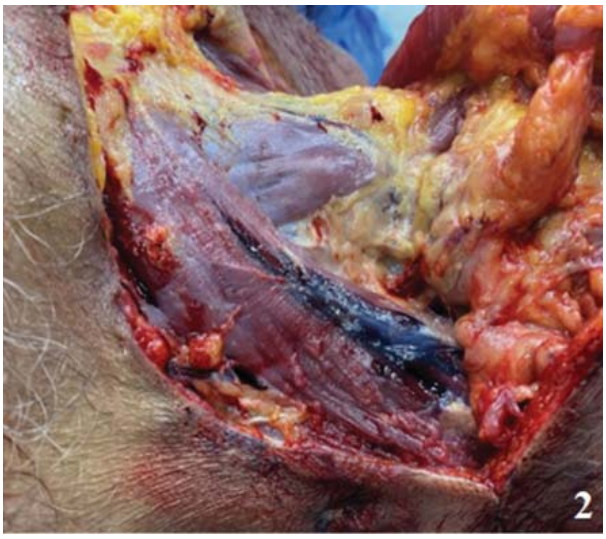


Fig. 2: External hemorrhage seen on Traditional autopsy.

Conventional autopsies may overlook or miss some hyoid or thyroid fractures. PMCT non invasively, adds information regarding the cartilaginous as well as bony structural lesions and abnormalities which is a definite merit of using CT.[2,11] Many times anatomical variations of the larynx make it extremely difficult to detect fractures not only on traditional autopsy but also on PMCT. Schulze et al., in his study described the detection of gas bubbles along with the fractures in laryngohyoid complex.[16] The incidence of thyroid and hyoid fractures in Indian population is less which is evident in our study and the studies done previously as well.[4,14] This can be attributed to the nature of ligature material used, as harder the material the greater would be increased local application of pressure over a small surface to the internal neck structures resulting in fractures.

In addition, the age of the subject plays an important role in defining the presence and absence of fracture, as younger individuals have slender built so more force is required for the same to occur. In our study the age group of more than 60 years was present in 2 cases, out of which both had fractures. Increased age causes ossification of the laryngeal structures, thereby increasing the chances of hyoid and thyroid fractures.[18,19] Due top revalence of sexual dimorphism, increased incidence of fractures in males compared to femalesis established in the literature and the same is evident. Hence the authors here report the presence of both fracturesin

male subjects in concordance with the literature.[19] According to Charoonnate et al, fractures of the hyoid and thyroid were present in 25% of the Thai population, and the incidence was more in the older age group.[20] Thali et al in 2003 detected hyoid fracture in 100% of its cases. According to a review by D Gascho et al out of a total of 26 hyoid fractures found in 126 cases, PMCT was able to detect 23 of them.[13] In the present study the authors were able to find a unilateral greater horn fracture of hyoid in a 65-year-old male in both modalities (fig. 3A & 3B). A bilateral thyroid cartilage fracture was found in one case and is well appreciated in both PMCT as well as traditional autopsy (fig. 4A & 4B). In addition to this, one more fracture of thyroid was found on CT which could not be appreciated at traditional autopsy. Thesyudy of D Gascho et al showed that the thyroid fracture in 25 cases was found in CT as well, only one case was midde.[13]

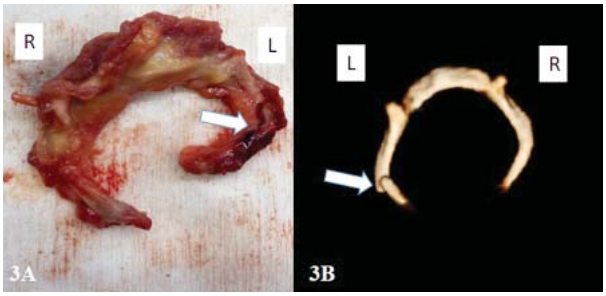


Fig. 3a: Unilateral hyoid fracture on Traditional autopsy.

Fig. 3b: Unilateral hyoid fracture on 3D reconstructed image of Hyoid.

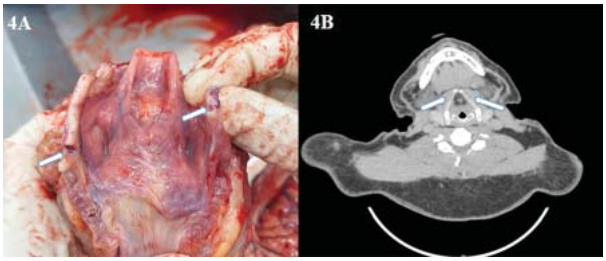
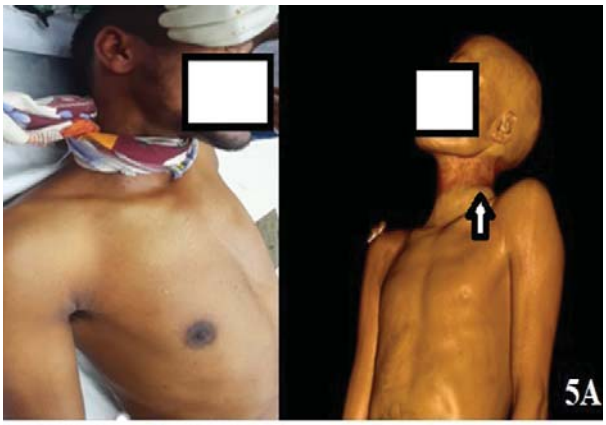


Fig. 4a: Bilateral Thyroid fracture on Traditional autopsy.

Fig. 4b: Bilateral Thyroid fracture on Axial CT.

Apart from the age of the deceased, suspension point, the type of hanging (in terms of complete and partial) and ligature material also plays an important role in extent of internal neck injury. In this study the most common ligature material used was soft cotton cloth in the form of bedsheet or dupatta (shawl) (fig. 5A & 5B). Sharma et al.[21], Ahmad and Hussian[22] and Patel et al.[23] have reported the chunni/dupatta (soft cotton cloth) as the most common ligature material followed by the rope.



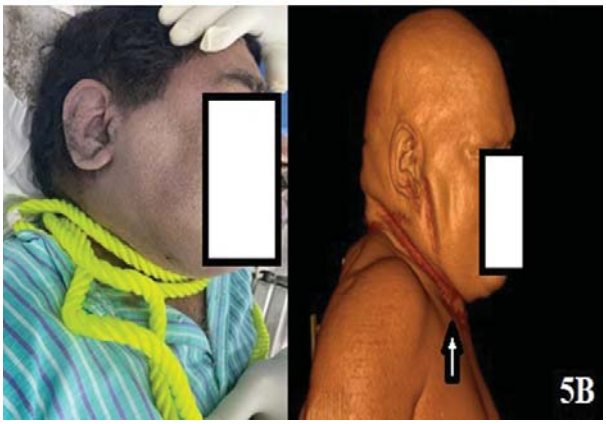


Fig. 5a: Traditional and 3D reconstructed CT image showing bedsheet as ligaturematerial.

Fig. 5b: Traditional and 3D reconstructed CT image showing rope as the ligaturematerial

In contrast Tumram et al reported nylon rope as the commonest ligature material.[10] As a norm, in routine practice, hanging is always considered suicidal in manner till the contrary is proved.[24] In many jurisdictions, where Medical Examiner's or Coroner's systems of the inquest are in practice, the law allows handing over the deceased body without dissection if the preliminary investigation revealed no foul play.[25] PMCT is being used in many of these jurisdictions to avoid unnecessary dissections in cases with no possibility of future litigation.[25] In India, Section 174 of The Code of Criminal Procedure 1973 is a procedural law that categorizes deaths that need a preliminary investigation by the Police. The law directs the investigating officer to take the body to an empowered doctor for examining the body in all unnatural death cases and in cases without a medically certified cause of death. Thus, through usage PMCT in confirming the cause of death in combination with a detailed external examination, in nonsuspicious hanging cases, unnecessary internal dissection can be avoided.

RECOMMENDATIONS

Firstly, this study has a sample size of fifty, and the incidence of fractures of hyoid and thyroid is less in Indian population. Therefore, taking a larger sample size will further validate the results of the study. Secondly, this study is based on the concept of taking traditional autopsy as the gold standard. However, there are studies done previously which prove PMCT is better than Traditional autopsy in certain cases. Authors would suggest using a combination of thorough external examination and ruling out the possible internal injuries due to other causes like firearm, injuries due to blunt trauma etc at PMCT is sufficient enough to conclude a case of hanging. Even the instances where the presence of fracture in PMCT alone, was taken as falsely positive tarhetted examination of checking the thyroid under the electron microscope could solve the purpose. Improvising in these aspects in future studies would authenticate the findings.

CONCLUSIONS

There is a great degree of concordance in the findings of the PMCT with that of traditional autopsy in the case of skin, subcutaneous tissue desiccation, thyroid fracture, and hyoid fracture. Authorsfurher intend to explore the implications of PMCT angiography for the identification of vessel injuries and resultant soft tissue haemorrhages, which would help in concluding nonsuspicious cases of hanging in a minimally invasive way.

LIST OF ABBREVIATIONS

PMCT: Post-mortem Computed Tomography

MSCT: Multi-Slice Computed Tomography

CT: Computed Tomography

ICC: Interclass correlation

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Forensic Chemical Profiling of Hazardous Additives and Contaminants alongwith their harmful Effects & Source discrimination of seized Moonshine samples: A study on New Emerging Crisis in Punjab

Deepak Middha[1], Archna Negi[2], Meenu Kushwaha[3]

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Abstract

Moonshine an illegally produced alcoholic beverage has caused several times "hooch tragedies" in various states of India. Punjab which is already facing Drug addiction hitch, has also not been untouched by hooch tragedies since 2020 when 120 people in Punjab died due to consumption of hooch. To curb hooch tragedies, Govt. of Punjab showed the very urgency for the inclusion of death penalty in Punjab & Excise Act, 1914. However, owing to shortfalls in quality control norms and non availability of a reliable facility to identify additives & contaminants in moonshine, avaricious manufacturers are still intentionally adding large number of hazardous additives and ignoring contaminants while manufacturing moonshine to make the brew strong and aromatic. More surprisingly, at present people are still considering methanol a root cause of hooch tragedies. Investigating agencies are more inquisitive to know the presence of methanol in seized samples to qualify them as 'unfit for drink'. It is crystal clear that merely the presence of methanol in any sample does not prove it lethal as it has also been studied that many alcoholic beverages contain methanol naturally at low level without causing harm. At present, a study on hazardous additives and contaminants in moonshine alongwith their harmful effects & source discrimination has not yet been reported. Henceforth, a forensic attempt is made to secure public health by chemically profiling various hazardous additives and contaminants detected in seized moonshine samples alongwith enlightening their harmful effects on body so as to make public aware of the hidden health hazards of moonshine. In this study, 31 samples of moonshine seized by Punjab Police from various districts of Punjab were extracted, sonicated and analysed by GCMS technique for the detection of hazardous additives and contaminants. For comparison & discrimination of source of these samples, grouping of detected chemicals was done using Principal Component analysis (PCA) a cluster analysis method. Our research outcome led to successful chemical profiling of 187 detected chemicals and among these 187 chemical compounds, 73 chemical compounds were found hazardous as these can cause severe health problems viz. eye, nose & throat irritation, inflammation to monocytes, birth defects, CNS disorder, cancer & death. Hence through this research an effort is made to secure public health and to alert govt. agencies regarding these health hazardous additives and contaminants.

Keywords: Moonshine; Hooch; Forensic; Additives; Contaminants, Death; hazardous; Public health etc.

Author Affiliation: [1]Deputy Director, [2]Junior Scientific Officer, (Chemistry), [3]Forensic Professional, Central Forensic Science Laboratory, Directorate of Forensic Science Services, Government of India, Chandigarh 160036, Punjab, India.

Corresponding Author: Archna Negi, Junior Scientific Officer (Chemistry), Central Forensic Science Laboratory, Directorate of Forensic Science Services, Government of India, Chandigarh 160036, Punjab, India.

E-mail: archnaforensic@yahoo.com

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INTRODUCTION

Alcoholic beverages have been used in Indian societies since the Vedic era (1500-700 BCE). It can be produced locally or industrially with the available resources across the world. The raw materizals generally used for the production of alcohol are fruits, palm wine, sugar cane etc. and the basic method used for its production is fermentation & simple distillation. At present alcohol is all pervasive and the mode of its intake has been constantly evolving. In India, alcohol consumption is widespread across all the states & the union territories and an estimated 160 million people consume alcohol.[1] The Indian Govt. has made multiple efforts to limit the availability of alcohol in pursuit of various public health objectives. But the problem does not cease here as most of the bootleggers are selling "moonshine" also known as 'hooch', a cheap alcoholic drink manufactured in small unregulated shanties to the poor population and in states that have imposed a full ban on liquor.

It is evident that the presence of methanol 'a toxic alcohol' in hooch has caused several times "hooch tragedies" in village population and urban poor in several states of India. A bar chart showing mortality v/s states due to hooch tragedies in India since 2008 to 2020 is depicted in fig. 1.

Mortality v/s States

Surprisingly, it has been observed that in the last

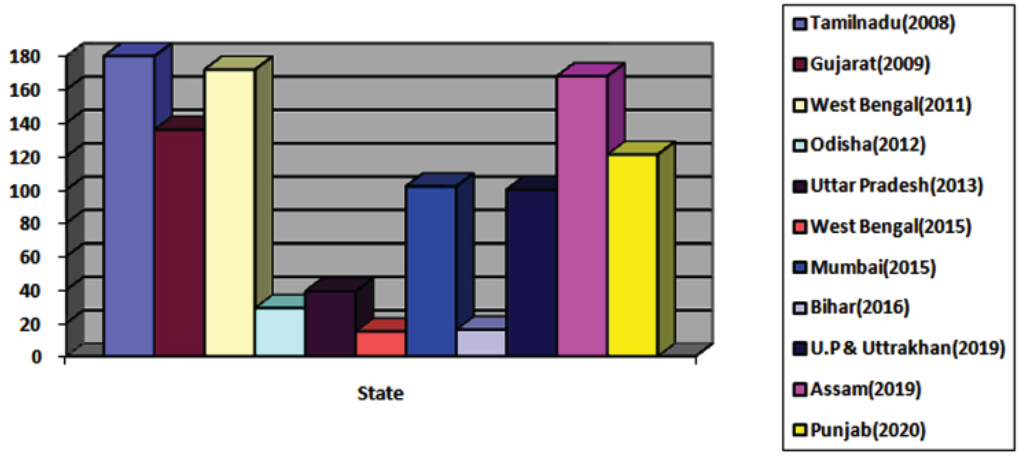


Fig. 1: Bar chart depicting Mortality due to hooch tragedies in various states of India.

few years, Punjab is disgraced having rise of **drug addled** persons especially youth. It has also not been untouched by hooch tragedies and has been facing new crisis "***Moonshine illicit liquor***". Most of the drug peddlers in Punjab have changed their business from drug smuggling to "bootlegging". Such mafias have no longer apprehension for prosecution and are now more intrepid in adding large number of harmful adulterants to make brew stronger i.e. lizard skins, alprazolam tablets and Iodex.[2] The year, **2020 was the pandemic year** for the people of Punjab when 120 people in the districts of Amritsar, Gurdaspur and Tarn Taran died due to consumption of hooch. To occlude this, Punjab police have raided different places in Punjab and large amount of hooch was seized. This incident trembled the Govt. of Punjab and showed the very urgency for the inclusion of ***death penalty***[3] in Punjab & Excise Act, 1914 in the year 2021 to curb hooch tragedies. Besides, the Hon'ble Punjab & Haryana High Court ordered an iron-hand approach for dealing these cases[4]; Punjab police added Section 302 (murder) of IPC in cases against all the kingpins of hooch tragedies and Punjab Excise Commissioner issued guidelines for transportation of Ethyl Neutral Alcohol, Ethanol, Specially Denatured Spirit, Denatured Spirits, and Rectified Spirit. Despites tightening its noose against bootleggers, the cases of hooch tragedy have not been ceased in Punjab. However, owing to shortfalls in quality control norms and non availability of a reliable facility to identify additives & contaminants in moonshine, avaricious manufacturers are still intentionally adding large number of hazardous additives and ignoring contaminants while manufacturing moonshine to make the brew strong and aromatic. These hazardous additives includes organic solvents & flavouring chemicals and pesticides like contaminants. The intake of organic solvents can cause severe health problems viz. eye, nose & throat irritation, CNS disorder, seizures & death.[5] Flavouring additives can cause inflammation to monocytes, cancer, birth defects, neurotoxic, CNS disorder & allergic reactions.[6] Pesticides additives have effect on reproduction, immune or nervous system, can cause cancer.[7] It

has also been studied that the solution obtained after distillation with a minimum ethanol content contains trace amount of methanol, esters, aldehydes, higher alcohols, acetates, acetic acid and fusel oil. Like methanol, some of these chemical compounds are also harmful. More surprisingly, at present people are still considering methanol a root cause of hooch tragedies. Investigating agencies are more inquisitive to know the presence of methanol in seized samples to qualify them as moonshine 'unfit for drink'. It is crystal clear that merely the presence of methanol in any sample does not prove it lethal as it has also been proven that many alcoholic beverages contain methanol naturally at low level without causing harm.[8] Its maximum legal concentration in different alcoholic beverages has already been defined by Food Safety and Standards (Alcoholic Beverages) Regulations, 2018. Till date only determination of methanol and its percentage in moonshine have been published so far. Several methods like spectroscopic, colorimetric etc. have been used for determination of methanol content in alcoholic drinks.[9-11] A study on hazardous additives and contaminants in moonshine alongwith their harmful Effects and source discrimination has not yet been reported.

Henceforth, a forensic attempt is made to secure public health by chemically profiling various hazardous additives and contaminants detected in seized moonshine samples alongwith enlightening their harmful effects on body so as to make public aware of the hidden health hazards of moonshine. In this study, 31 samples of moonshine were subjected to forensic chemical analysis. These samples were seized during raids conducted in different parts in Punjab by Punjab police under Excise Act and were submitted at Central Forensic Science Laboratory, Chandigarh, for the detection of Methanol. Various laboratory examinations such as Physical tests, Chemical tests, Gas Chromatography analysis and Gas Chromatography Mass Spectrometric analysis (GC-MS) were carried out with these samples. Ethanol was detected in 05 seized samples while Methanol was detected in 24 seized samples. The percentage of ethanol and methanol was determined using specific gravity and calibration graph method respectively. Apart from this, 187 additives including contaminants were identified by mass spectral chemical profiling. For comparison and discrimination of source of these samples, grouping of 187 detected chemicals was done using ***Principal Component analysis (PCA)*** a cluster analysis method.

MATERIALS AND METHODS

In this research paper, thirty one (31) seized samples of various moonshine samples were subjected to forensic chemical analysis. These samples were seized (under Excise Act) during a regular search in various district of Punjab u/s 61/78(2) Punjab Excise Act,1914 & 420/468/471 IPC 1860 by Punjab Police and submitted at Central Forensic Science Laboratory, Chandigarh for chemical analysis. All of the submitted plastic nips (quarters) were found without printed label of any brand.

The representative samples taken from 31 seized moonshine samples were tested for the detection of ethanol, methanol, copper, iron & furfural. Specific gravity method was used for estimating ethanol percentage and calibration graph method was used for methanol concentration. For complete chemical profiling of hazardous additives and contaminants in representative samples of seized moonshine samples were then analyzed by gas chromatography mass spectrometry (GC-MS) technique.

The Solvent used for extraction was of LC grade (Merck, German).

Fig. 2: Samples sized from various district of Punjab

Extraction of seized moonshine samples for additives and contaminants

10 ml of each seized samples were extracted three times with 10 ml chloroform by liquid-liquid extraction procedure. These chloroform extracts were combined in china dish, was concentrated and stored at 40^0C.

Equipment

Shimadzu Nexis GC2030 coupled with Shimadzu QP2020 NX MS and Shimadzu autosampler AOC-20i Plus were used.

INSTRUMENTATION CONDITIONS

Shimadzu, QP-2020NX

Shimadzu Nexis GC2030 coupled with Shimadzu QP2020 NX MS and Shimadzu autosampler AOC-20i Plus were used for chemical profiling. The GC-column was a 30m SH-Rxi-5Sil MS with 0.25 mm I.D. & 0.25µm film thickness. Helium was used as a carrier gas at a constant flow of 1 ml/min. Splitless injection was used with a splitless time of 60s. The injector & interface line temperature were held at 250^0C & 330^0C respectively. Oven initial temperature was set at 60^0C for 2 minute; increased to 80^0C at the rate of 6^0C/min & hold at this temperature for 4 minutes and then increased to 250^0C at the rate of 10^0C & held at this temperature for 2 min.

The MSD conditions: - Ionisation energy 70 eV, ion source temperature 200^0C, mass range 50-500 amu, electron multiplier voltage (Auto tune + 200V).

Sample injection volume: - 1 µl.

Compound Identification.

MS Real Time Analysis software was used for data acquisition & processing and the result(s) were scrutinized via MS-library of National Institute of Standard and Technology..

RESULTS

Various laboratory examinations such as Physical tests, Chemical tests and Specific gravity measurements were carried out with the 31 seized samples and are summerised in Table 1.

In addition to physical and chemical analysis, percentage of ethanol was calculated in sample 27

Table 1: Tests performed with seized samples

Sample No.	Physical Tests			Chemical Tests				
	Volume (approx)	Colour	Odour/ Aroma	Iodoform test for Ethanol	Dichromate test for Ethanol	Aniline test for Furfural	Chromo-tropic acid test for Methanol	Potassium ferrocyanide test for Copper and Iron
1 to 24	Each 180 ml	all having colourless liquid	all having pungent odour	-VE in all samples	-VE in all samples	-VE in all samples	+VE in all samples	-VE in all samples
25	180 ml	Colorless liquid	Sweet aroma	-VE	-VE	-VE	-VE	-VE
26	180 ml	Yellow coloured liquid	Sweet fruity aroma	-VE	-VE	-VE	-VE	-VE
27	180 ml	Caramel coloured liquid	Sweet fruity aroma	+VE	+VE	-VE	-VE	-VE
28	180 ml	Light yellowish liquid comprising with oil type suspension	Sweet fruity aroma	+VE	+VE	-VE	-VE	-VE
29	180 ml	Slight yellowish liquid comprising two immiscible liquid layers.	Sweet fruity aroma	+VE	+VE	-VE	-VE	-VE
30	180 ml	Dark brown coloured thick viscous material	Burnt molasses odour	+VE	+VE	-VE	-VE	-VE
31	180 ml	Dark brown coloured thick liquid	odour alike of medicinal syrup	+VE	+VE	-VE	-VE	-VE

to 29 using specific gravity method, methanol was quantified in sample 1 to 24 by calibration graph method using GC technique and for identification of various hazardous compounds, chloroform extracts of seized moonshine samples were analyzed by GC-MS technique. The percentage of methanol in Exhibit-1 to Exhibit-24 was found as 0.77, 0.81, 0.93, 0.69, 0.57, 0.8, 0.77, 0.77, 0.77, 0.77, 0.79, 0.78, 0.76, 0.77, 0.8, 0.77, 0.78, 0.73, 0.76, 0.75, 0.82, 0.78, 0.81, 0.83 respectively; percentage of ethanol in Exhibit-27 to Exhibit 29 was found as 0.07, 0.26 & 0.07 respectively (ethanol detected in traces in Exhibit 30 & Exhibit 31) and 187 chemical compounds were detected and identified in seized sample 1 to 31. These detected chemicals according to their nature were categorized as Phytochemical compounds; Pyrolysis byproducts; Fermentation byproducts; Pesticides; Organic solvent; Flavouring substances/ Essential oils & Antioxidant compound. The resulting total ion chromatograms (TICs) are depicted in Fig. 3 the detected chemicals are tabulated alongwith Rt & molecular structure in table 2.

Grouping study by statistical methods using identification of chemicals detected in seized moonshine's samples

For comparison and discrimination of source of these detected chemicals, grouping was done using cluster analysis methods namely ***Principal Component analysis (PCA)*** as depicted in Fig. 4.

DISCUSSION

Table 2: Chemical profiling of chemical compounds detected compounds in seized moonshine samples.

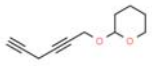
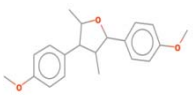
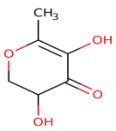
S.No.	Chemical compounds	Exhibits	Molecular structure	Rt
1.	**Phytochemical compounds**			
I.	**Heterocyclic compound**			
(i)	1,3,5,7-Tetroxane	4, 6, 9 to12, 14 to 18 and 20 to 24.		2.155, 2.910, 2.865, 2.130, 2.42, 2.750, 2.045, 2.880, 2.045, 2.22, 2.110, 2.045, 2.605, 2.405, 2.930, 2.020, 2.515
(ii)	2H-Pyran,2-(2,5-hexadiynyloxy) tetrahydro	13, 17 and 18		1.770, 1.665, 1.650
(iii)	3-Dodecyl-2,5-furandione	26		26.67
(iv)	Furan, tetrahydro-2,4-bis(4-methoxyphenyl)-3,5-dimethyl	26		17.955
(v)	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	30		12.490

Table contt...

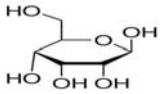
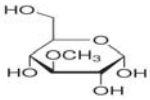
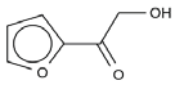
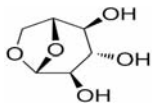
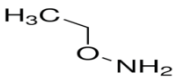
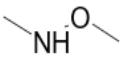
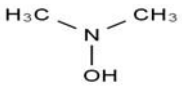
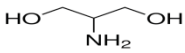
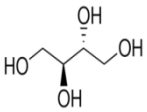
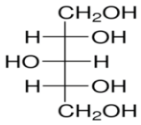
II.	**Bicyclic compound**			
(i)	Thujene	28		6.725
III.	**Monosaccharide**			
(a)	**Aldohexoses**			
(i)	D-Allose	30		19.435
(ii)	3-O-Methyl-d-glucose	30		16.140
IV.	**Hydroxy Ketone/ Hydroxyacetone/ Acetol**			
(i)	Furyl Hydroxymethyl ketone	30		10.430
2.	**Chemical compounds formed during pyrolysis of carbohydrates, such as starch and cellulose**			
a.	**Cyclic Ketohexoses**			
(i)	1,6-Anhydro-.beta.-Dglucofuranose/ Levoglucosan (furanose)	30 &31		20.920 & 20.730
3.	**Chemical compounds formed during fermentation**			
I.	**Nitrogen compound**			
a.	**Amine: Amines occur in liquor due to the biochemical degradation of amino acids, which may begin during malting and then continues during fermentation.**			
(i)	O-Ethylhydroxylamine	1, 4 to 7, 9 to 12, 14 to 22 and 24.		2.345, 1.82, 2.955, 3.09, 2.93, 2.385, 2.56, 2.055, 2.625, 2.235, 2.31, 2.45, 2.485, 3.005, 2.555, 2.495, 2.825, 2.010, 2.170
(ii)	Methanamine N-methoxy	1, 2, 8, 15, 20 and 22 to 24 & 31		4.495, 1.62, 1.905, 1.940, 1.945, 2.095, 2.480, 3.710, 13. 500
(iii)	Methanamine, N-hydroxy-N-methyl/ N,N-Dimethylhydroxylamine	20, 22 and 23.		2.375,2.39, 2.35
II	**Amino alcohol**			
(i)	Serinol/aminoglycerin/ 2-amino-1,3-propanediol	23		3.840
III	**Sugar alcohol**			
(i)	Erythritol	1, 2 and 4 to24		4.630, 4.790, 5.12, 13.55, 4.72, 4.57, 4.615, 4.845, 4.695, 4.975, 4.605, 4.575, 3.52, 4.925, 5.205, 4.9, 4.970, 4.650, 4.460, 4.87, 5.770, 4.820, 5.020 , 4.720
(ii)	Xylitol	7		3.300

Table contt...

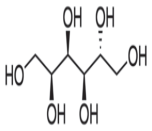
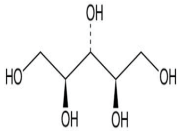
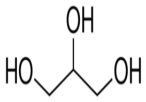
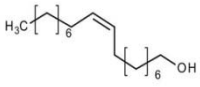
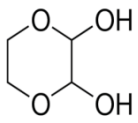
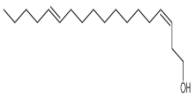
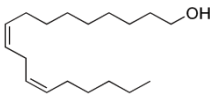
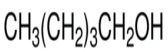
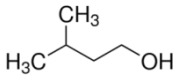
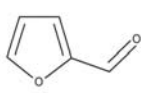
(iii)	Sorbitol	13		2.95
(iv)	Ribitol	23		5.820
(v)	Glycerin	1, 2 and 4 to 24 & 31		3.22, 4.945, 5.455, 5.47, 5.27, 2.725,3.195, 2.265, 2.265, 2.24, 2.425, 2.03,2.07, 2.105, 1.67, 2.260, 2.385, 5. 675,5.895, 5.42, 5.145, 5.495, 4.895
IV.	**Alcohol: Alcohols produced by reduction of acids/ fermentation**			
(i)	9-Octadecen-1-ol, (Z)	3		22.57
(ii)	Dioxanediol/ 2,3-Dihydroxy-1,4-dioxane	1, 3 to 7, 9 to 22 and 24		4.82, 1.585, 1.615, 1.65, 1.62, 1.615, 2.265, 1.655, 3.175, 3.275, 3.055, 2.215, 3.995, 2.895, 3.905, 2.35, 3.015, 2.245, 2.895, 5.115, 2.210, 2.040, 2.120
(iii)	E,E-3,13-Octadecadien-1-ol	2		23.77
(iv)	9,12-Octadecadien-1-ol, (Z,Z)/ Linoleyl alcohol	6		25.065
(v)	1-Butanol	27		1.93
(vi)	1-Pentanol/ n-Butylcarbinol/ amyl alcohol	27		4.15
(vii)	Isoamyl alcohol/ Fermentation amyl alcohol/ Fusel oil	27		3.095
V	**Hydrocarbon**			
(i)	8-Heptadecene	2 and 8		21.45 & 21.45
VI.	**Aldehyde**			
(i)	Furfural	30		3.850

Table contt...

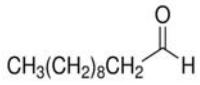
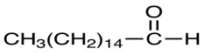
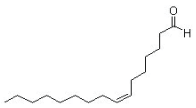
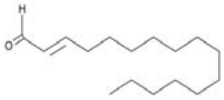
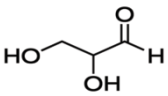
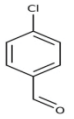
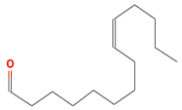
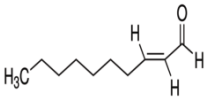
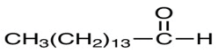
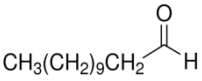
(ii)	Undecanal	28		16.050
(iii)	Hexadecanal	1 to 4 and 19		23.115, 23.115, 23.12, 23.12, 23.115, 23.130
(iv)	7-Hexadecenal (Z)	2, 3, 7,8, 10, 16 and 19		22.865, 23.83, 25.085, 22.865, 27.385, 25.105 & 25.08
(v)	9-Hexadecenal(Z)	2 & 28		26.500
(vi)	E-Hexadec-2-enal/ trans-2-Hexadecenal	8 and 20		17.025, 17.055
(vii)	Glyceraldehyde	10, 15, 20 and 22 to 24 & 30		2.53, 1.655, 3.405, 2.295, 2.540, 5.680, 1.905
(viii)	Myristaldehyde	2		20.645
(ix)	Benzaldehyde, 4-chloro	1		15.195
(x)	9-Octadecenal	1, 8 and 19.		23.83, 23.825, 24.005
(xi)	9-Tetradecenal	1 and 3		26.820 & 27.365
(xii)	2-Decenal(E)-/ trans-2-Decenal	19		15.14
(xiii)	Pentadecanal	2, 8 and 20		21.915, 21.915 & 25.325
(xiv)	Dodecanal/ Lauraldehyde	28		17.745

Table contt...

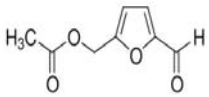
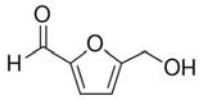
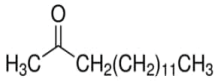
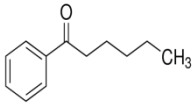
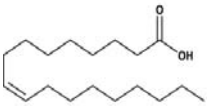
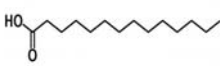
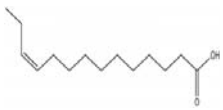
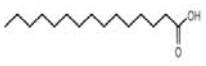
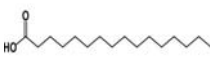
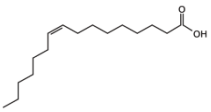
(xv)	5-Acetoxymethyl-2-furaldehyde	30		15.995
(xvi)	5-Hydroxymethylfurfural / HMF	30 & 31		14.745 & 14.490
VII.	**Ketone**			
(i)	Methyl tridecyl ketone/ Pentadecan-2-one	2		21.705
(ii)	Linoleyl methyl ketone	3, 4 and 19		25.665, 25.665 & 25.730
(iii)	Hexanophenone	2		18.585
VIII.	**Acids/volatile acids**			
(i)	Oleic acid/ 9-Octadecenoic acid(Z)	1 to 21 & 30		26.485, 26.7, 26.74, 26.59, 24.745, 26.57, 26.595, 26.595, 26.545, 26.57, 26.555, 26.595, 24.77, 26.6, 26.585, 26.615, 26.64, 26.670, 26.625, 26.630, 26.74, 26.410
(ii)	Elaidic acid / 9-octadecenoic acid(E)	1 & 2		26.53 & 26.7
(iii)	Acetic acid	1 & 23		4.825 & 2.075
(iv)	Dodcanoic acid	2		20.015
(v)	Tetradecanoic acid	2		22.495
(vi)	Z-11-tetradecenoic acid	2		22.365
(vii)	Pentadecanoic acid	2		23.62
(viii)	n-Hexadecanoic acid	1, 2, 29 & 30		24.665, 24.83, 24.735 & 27.67
(ix)	9-Hexadecenoic acid,(Z)/ Palmitoleic acid	2		24.565

Table contt...

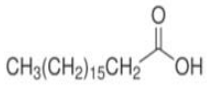
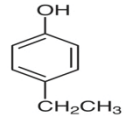
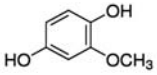
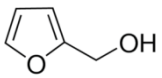
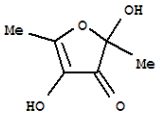
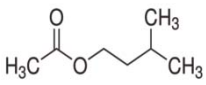
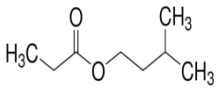
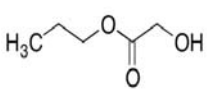
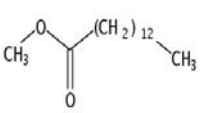
(x)	Octadecanoic acid	1		26.72
IX.	**Phenolic compound**			
(i)	p-Ethylphenol	31		13.135
(ii)	2-Methoxyhydroquinone	30		15.820
X	**Furans**			
(i)	Furfuryl alcohol	30		4.150
(ii)	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	30		6.765
XI.	**Ester: These are formed by the reactions of organic acids and alcohols created during fermentation.**			
a.	**Acetate ester**			
(i)	Linoleyl acetate	3, 4 & 9		25.065, 25.065 & 25.05
(ii)	7-Dodecen-1-yl-acetate	2 & 8.		21.355 & 21.36
(iii)	Isopentyl alcohol, acetate/ Isoamyl acetate	27		4.615
b.	**Propionate ester**			
(i)	Isopentyl alcohol, propionate/ Isoamyl propionate	27		6.645
c.	**Glycolate ester**			
(i)	Propyl glycolate	3		2.01
d.	**Fatty acid methyl ester**			
(i)	Methyl tetradecanoate/ Myristic acid, methyl ester /Tetradecanoic acid, methyl ester	2 and 3		22.0 & 22.01
(ii)	Methyl Palmitoleate/ 9-Hexadecanoic acid, methyl ester (Z)	2, 3 and 8		24.055, 24.06 & 24.05

Table contt...

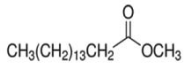
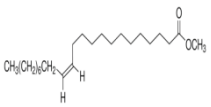
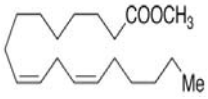
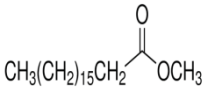
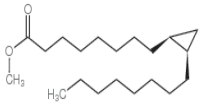
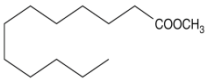
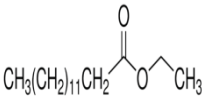
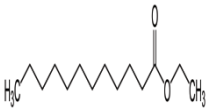
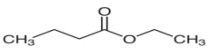
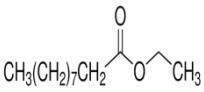
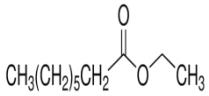
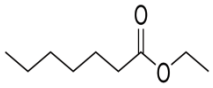
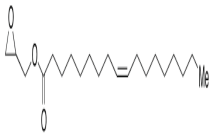
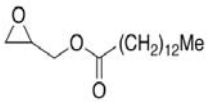
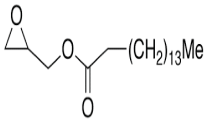
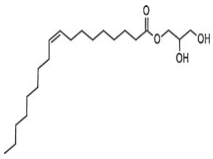
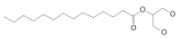
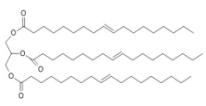
(iii)	Palmitic acid, methyl ester/ Hexadecanoic acid, methyl ester	1 to 4, 8, 10 and 19.		24.28, 24.28, 24.28, 24.28, 24.28, 24.285 & 24.280
(iv)	13-Docosenoic acid, methyl ester/ methyl, 13-docosenoate	1, 3, 4, 7 and 8.		25.96, 25.95, 25.945, 25.95 & 25.945
(v)	6-Octadecenoic acid, methyl ester (Z)	1		25.33
(vi)	9-Octadecenoic acid methyl ester (E)/ Methyl elaidate	1 to 4, 6, 8 to 14, 17 and 19 to 21		26.1, 26.105, 26.1, 26.1, 26.1, 26.15, 26.1, 26.11, 26.115, 26.125, 26.115, 26.115, 26.11, 26.105, 26.100, 26.110
(vii)	9, 12-Octadecadienoic acid (Z,Z)-, methyl ester/ Methyl linoleate	2 to 4, 8, 9 and 15		26.035, 26.035, 26.035, 26.03, 26.98 & 26.985
(viii)	Methyl stearate/ Octadecanoic acid, methyl ester	2 to 4, 8 and 17		26.345, 26.345, 26.345, 26.34 & 26.36
(ix)	Cis-9,10-Methyleneoctadecanoic acid, methyl ester	3		25.95
(x)	Dodecanoic acid, methyl ester/ Metholene 2296	27		19.405
e.	**Fatty acid ethyl ester**			
(i)	Myristic acid, ethyl ester/ Ethyl myristate	27		22.795
(ii)	Dodecanoic acid, ethyl ester/ Ethyl laurate	27		20.355
(iii)	Ethyl butanoate /Butanoic acid, ethyl ester	26 & 27		3.075 & 3.165
(iv)	Ethyl caprate/ Decanoic acid, ethyl ester	27		17.5

Table contt...

(v)	Ethyl caprylate/ Octanoic acid, ethyl ester	27		13.775
(vi)	Ethyl enanthate/ Heptanoic acid, ethyl ester	27		11.085
f.	**Fatty acid glycidal ester**			
(i)	9-octadecenoic acid(Z)-oxiranylmethyl ester/ glycidol oleate/ glcidyl octadecenoate/ oleic acid, glycidyl ester	1 to 6, 8 to 14, 17 and 20		25.225, 28.02, 25.225, 25.215, 25.215, 25.215, 25.205, 25.22, 25.21, 25.21, 25.215, 25.23, 25.22, 25.22, 25.23, 25.20
(ii)	Tetradecanoic acid, 2-oxiranylmethyl ester/ Myristic acid glycidyl ester	4, 5 and 8		25.875, 25.89 & 25.895
(iii)	Hexadecanoic acid, 2-oxiranylmethyl ester/ Glycidyl palmitate/ Oxiran-2-ylmethyl palmitate	3, 4, 6, 8 and 13		25.875, 28.235, 28.235, 28.235 & 25.895
g.	**Fatty acid glycerol ester**			
(i)	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester/ Palmitin, 2-mono	3, 4, 6, 8, 9, 10, 13,14, 17, 19, 20		27.66, 27.66, 27.665, 27.67, 27.66, 27.680, 27.67, 27.69, 27.675, 27.66 & 27.65
(ii)	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester/ Glyceryl monooleate	3, 4, 5, 8, 10, 13, 14, 17, 19		23.67, 23.575, 27.445, 23.67, 23.68, 23.67, 23.68, 23.69, 23.695, 23.695 & 23.69
(iii)	Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester/ Myristin, 2-mono	4		25.55
(iv)	9, 12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester/ Linolein, 2-mono	3 & 4		23.395 & 23.44
(v)	9,12-Octadecadienoic acid (Z,Z)-, 2,3 dihydroxypropyl ester/ Alpha-glyceryl linoleate	3		23.44
(vi)	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)/ glycerine trioleate	4 & 5		24.555 & 26.58

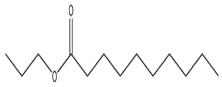
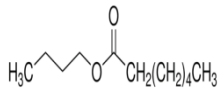
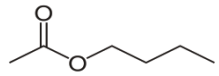
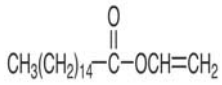
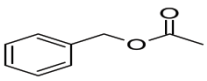
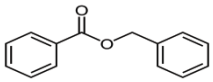
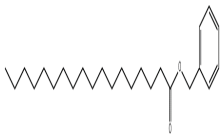
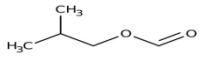
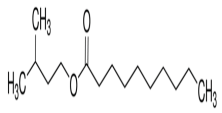
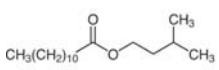
h.	**Fatty acid propyl ester**			
(i)	Dodecanoic acid, propyl ester/ Propyl decanoate	21		4.900
i.	**Fatty acid butyl ester**			
(i)	Oleic acid, butyl ester	19		1.495
(ii)	Butyl heptanoate/ Heptanoic acid, butyl ester	27		15.685
(iii)	Butyl ethanoate/ acetic acid, butyl ester	27		3.42
j.	**Fatty acid vinyl ester**			
(i)	Palmitic acid vinyl ester/ Hexadecanoic acid ethenyl ester	2 to 4		27.6, 27.58 & 27.58
k.	**Fatty acid benzyl ester**			
(i)	Acetic acid,phenylmethyl ester / Benzyl ethanoate / Benzyl acetate	19 & 27		12.93 & 12.945
(ii)	Benzyl Benzoate	1, 25 & 26		22.665, 23.8 & 22.65
(iii)	Octadecanoic acid, phenylmethyl ester/ Benzyl stearate	27		27.815
l.	**Fatty acid isobutyl ester**			
(i)	Formic acid, 2-methylpropyl ester	23		1.685
m.	**Fatty acid isoamyl ester**			
(i)	Pentadecanoic acid, 3-methylbutyl ester/ Isopentyl decanoate	27		21.01
(ii)	Dodecanoic acid, 3-methylbutyl ester/ Isopentyl laurate	27		23.385
(iii)	Tetradecanoic acid, 3-methylbutyl ester/ 3-Methylbutyl tetradecanoate	27		25.505

Table contt...

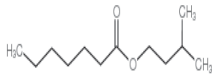
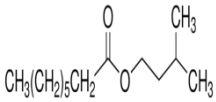
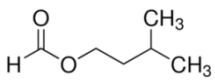
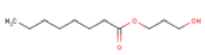
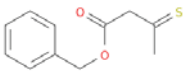
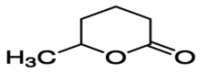
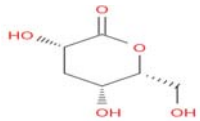
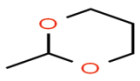
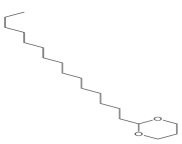
(iv)	Hexadecanoic acid, 3-methylbutyl ester/ 3-Methylbutyl hexadecanoate	27		27.555
(v)	Heptanoic acid, 3-methylbutyl ester/ Isopentyl heptanoate	27		16.745
(vi)	Octanoic acid, 3-methylbutyl ester/ Isoamyl caprylate	27		118.3
(vii)	Isoamyl formate	27		3.005
n.	**Fatty acid sabinyl acetate**			
(i)	Sabinyl linoleate/ Linoleic acid, sabinyl acetate	26		23.655
o.	**Fatty acid monohydroxy propyl ester**			
(i)	Octadecanoic acid, 3-hydroxypropyl ester	26		27.79
p.	**Fatty acid monohydroxy aryloxy ester**			
(i)	tert-Butyl 3-hydroxy-3-(4-methoxyphenyl) propanoate/ Propanoic acid, 3-hydroxy-3-(4-methoxyphenyl)-,t-butyl ester	25 & 26		19.155 & 22.965
q.	**Keto acids benzyl ester**			
(i)	Acetoacetic acid, 3-thio-,benzyl ester/ Benzyl 3-thioxobutanoate	26		12.43
r.	**Cyclic ester**			
(i)	delta.-Caprolactone	27		3.615
(ii)	3-deoxy-d-mannoic lactone	30		21.42
XII.	**ACETALS**			
(i)	1,3-Dioxane, 2-methyl	25		2.020
(ii)	1,3-Dioxolane, 4-methyl-2-pentadecyl	26		18.875
(iii)	1,3-Dioxane, 2-pentadecyl	26		26.01

Table contt...

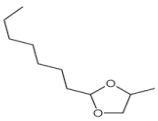
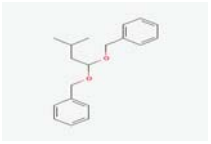
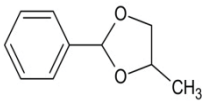
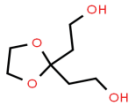
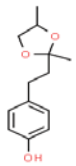
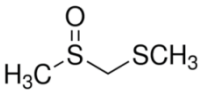
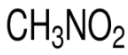
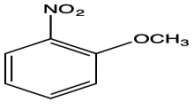
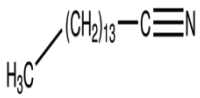
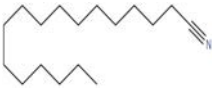
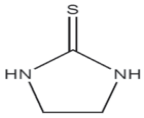
(iv)	1,3-Dioxolane, 2-heptyl-4-methyl	28 & 29		17.350 & 17.335
(v)	Isovaleraldehyde dibenzyl acetal	27		21.76
(vi)	Benzaldehyde propylene glycol acetal/ 1,3-Dioxolane, 4-methyl-2-phenyl	27		15.285
(vii)	1,3-Dioxolane-2,2-diethanol	26		28.22
(viii)	4-[2-(2,4-Dimethyl-1,3-dioxolan-2-yl) ethyl]phenol/ 4-(4-Hydroxyphenyl)-2-butanone propyleneglycol	26		22.77
XIII.	**Sulphur compound**			
(i)	Formaldehyde dimethyl mercaptal S-oxide	1, 2 and 4 to 24		1.665, 3.325, 2.045, 2.18, 2.02, 1.97, 1.645, 1.66, 2.05, 1.59, 2.275, 2.475, 2.945, 2.715,1.58, 2.23,1.99, 4.8, 1.875, 1.690, 1.875, 1.570 & 1.585
4.	**Pesticides/contaminants**			
I.	**Nitrogen compounds**			
a.	**Nitro: Pesticides/fuel additive/ agricultural products/explosive**			
(i)	Methane nitro	1 to 24 & 31		2.22, 1.545, 3.895, 1.915, 1.805 , 1.88, 2.055, 1.565, 2.2, 1.875, 2.105, 1.86, 2.35, 1.605, 1.59, 1.925, 1.970, 1.955, 1.780, 1.770, 1.765, 1.740, 1.765, 2.035 & 2.035
(ii)	Anisole, o-nitro/ Benzene, 1-methoxy-2-nitro/ o-Nitrophenyl methyl ether	16		3.790
b.	**Nitrile-agricultural products/pesticides**			
(i)	Pentadecanenitrile	1		22.88
(ii)	Heptadecanenitrile	1		25.125
c.	**Amide-** Fungicides/ Herbicides (contaminats)			
(i)	Ethylenthiourea	10 to 12, 18 and 20		5.520, 5.735, 5.425, 5.485 & 2.76

Table contt...

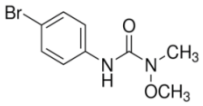
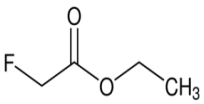
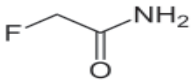
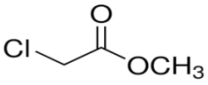
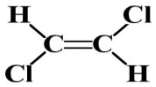
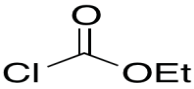
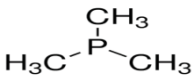
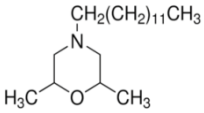
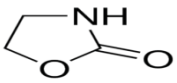
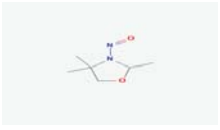
(ii)	Metobromuron/ Urea,N-(4-bromophenyl)-N-methoxy-N-methyl	13 and 20		3.775 & 2.76
II.	**Organofluorine compound: Rodenticide (contaminant)**			
(i)	Acetic acid, fluoro-, ethyl ester/ Ethyl monofluoroacetate/ Ethyl fluoroacetate	1, 3, 5 to 7, 9 to 14, 16, 17, 19 to 21 and 23		11.625, 4.995, 3.645, 17.135, 4.78, 2.505, 7.135, 3.58, 7.03, 3.685, 3.135, 3.705, 2.71, 4.245, 16.545, 5.18, 5.280
(ii)	Acetamide, 2-fluoro	5		7.375
III.	**Organochlorine compound (Pesticides/ Agrochemicals)**			
(i)	Methyl chloroacetate/ Acetic acid, chloro-, methyl ester	27		10.090
(ii)	Ethene,1,2-dichloro-,(E)/Trans-Di-1,2-Chloroethylene	30		3.795
(iii)	Carbonochloridic acid, ethyl ester/ Cathyl chloride/ Formic acid, chloro-, ethyl ester	30 & 31		1.405 1.385
IV.	**Organophosphorus compound (pesticide)**			
(i)	Trimethylphosphine	28, 29 & 31		2.615, 2.720, 2.905
V.	**Morpholine compound (Fungicide)**			
(i)	Tridemorph	30		10.525
VI.	**Oxazolidinones (Antimicrobials)**			
(i)	2- Oxazolidinone	28		17.550
(ii)	N-Nitroso-2,4,4-trimethyloxazolidine	30		16.275
5.	**Organic Solvent (Health hazardous chemical compounds)**			
I.	**Glycol ether**			
(i)	Carbitol/ Diethylene Glycol ethyl ether/ Ethanol, 2-(2-ethoxyethoxy)	25		11.165
(ii)	Diethyl carbitol	25		11.41

Table contt...

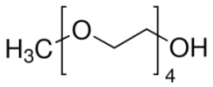
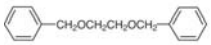
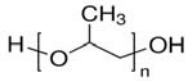
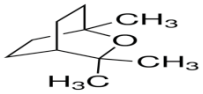
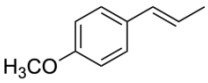
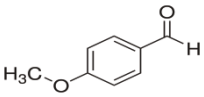
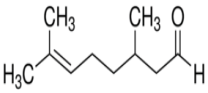
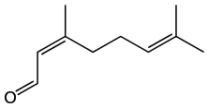
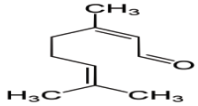
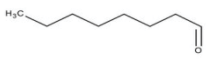
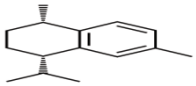
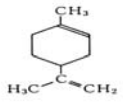
(iii)	Tetraethylene glycol, monoethyl ether	25		12.78,
(iv)	Ethane, 1,2-bis(benzyloxy)/ Ethylene Glycol Dibenzyl Ether/ Dibenzyl Glycol	25		24.82
(v)	Dipropylene glycol, butyl ether	28		11.650
(vi)	Ethylethylene glycol	28 and 29		6.275 & 10.030
(vii)	Polypropylene glycol/ 1-Propanol, 2-(2-hydroxy propoxy)	29		10.605 & 10.715
6.	**Flavouring substances/ Essential oils/Terpenoids**			
I.	**Ether**			
(i)	Eucalyptol	25		8.655
(ii)	Anethole	1 and 8 and 25		15. 695, 15.68 & 16.52
II.	**Aldehyde**			
(i)	p-Anisaldehyde	25		13.71
(ii)	Citronellal	28		12.650
(iii)	Citral	28 and 29		15.220, 15.180
(iv)	Neral	28 and 29		14.605 & 14.680
(v)	Caprylaldehyde/ Octanal	8 & 28		7.7007
III.	**Hydrocarbon**			
(i)	Cis-Calamenene	25		17.525
(ii)	D-Limonene	26, 28 & 29		10.12, 8.640 & 8.795

Table contt...

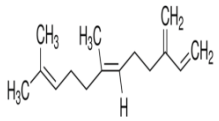
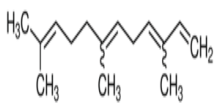
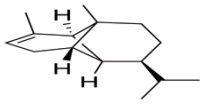
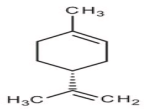
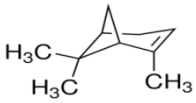
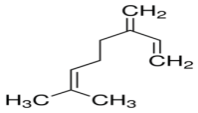
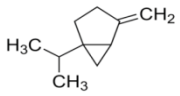
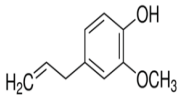
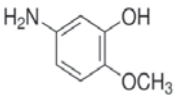
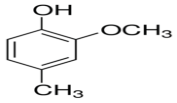
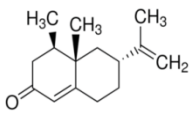
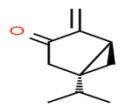
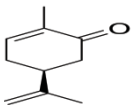
(iii)	(E)-.beta.-Farnesene	26		21.705
(iv)	alpha.- Farnesene	26		22.36
(v)	Copaene	26		17.305
(vi)	Limonene	27		8.530
(vii)	2-Pinene	28		5.675
(viii)	Myrcene/ beta-Myrcene	28 and 29		7.075 & 7.090
(ix)	Sabinene	28		6.725
(x)	n-Hexane	28 and 29		
IV.	**Phenolic compound**			
(i)	Eugenol	26 & 31		16.94 & 16.855
(ii)	o-Guaiacol/o-Methoxyphenol	31		10.645
(iii)	Creosol	31		13.595
V.	**Ketone**			
(i)	Nootkatone	26		23.17
(ii)	Sabinone	29		14.455
(iii)	D-Carvone	29		14.825

Table contt...

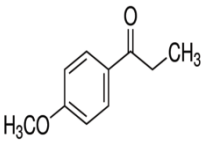
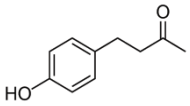
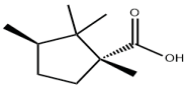
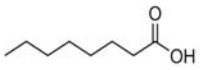
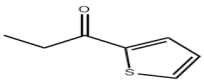
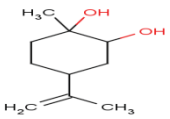
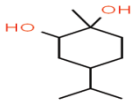
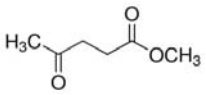
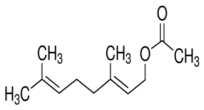
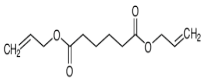
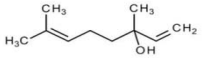
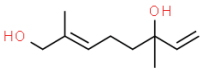
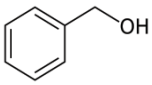
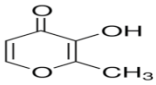
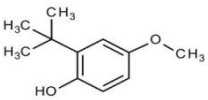
(iv)	1-Propanone, 1-(4-methoxyphenyl)/ Propiophenone,4-methoxy/ p-Methoxy propiophenone	25		18.510
(v)	2-Butanone, 4-(4-hydroxyphenyl) / raspberry Ketone	26		20.07
VI.	**Epoxide**			
(i)	Limonene oxide, trans	26		20.205
VII.	**Acids**			
(i)	n-Heptoic acid	27		11.81
(ii)	Oleic acid	26 , 27 & 30		26.470, 26.525 & 26.410
(iii)	n-Hexadecanoic acid/Palmitic acid	26, 27, 29 & 30		24.675, 24.700, 24.615 & 24.600
(iv)	D-Campholic acid	29		18.725
(v)	Octanoic acid	29, 30 & 31		13.685, 13.285 &13.305
VIII.	**Thienyl**			
(i)	Ethyl-2-thienylketone	30		12.645
IX.	**Cyclic vicinal glycol**			
(i)	1,2-Cyclohexanediol,1-methyl-4-(1-methylethenyl)/Limonene glycol	26 & 29		16.87 & 16.995
(ii)	Isocarvomenthol,1-hydroxy/ p-Menthane-1,2-diol	26		21.115

Table contt...

X.	**Keto acid**			
(i)	3-Methyllevulinic acid/ Pentanoic acid, 3-methyl-4-oxo	27		1.685
XI.	**Acetate ester**			
(i)	Acetic acid, geraniol ester/ Geranyl acetate	28		17.195
XII.	**Fatty acid geranyl ester**			
(i)	Geraniol butyrate/ Geranyl butyrate	28		16.900
XIII.	**Allyl Ester**			
(i)	Adipic acid, dially ester	30		20.425
XIV.	**Alcohol**			
(i)	Linalool	28 & 29		10.745 & 10.465
(ii)	alpha-Terpineol	28 & 29		13.335 & 13.865
(iii)	8-Hydroxylinalool			14.00
(iv)	Benzenemethanol/ Benzyl alcohol	31		8.730
XV.	**Hydroxypyranone**			
(i)	Maltol	30		11.430
7.	**Antioxidant compound**			
(i)	2-tert-Butyl-4-methoxyphenol/ 2-BHA	29		18.955

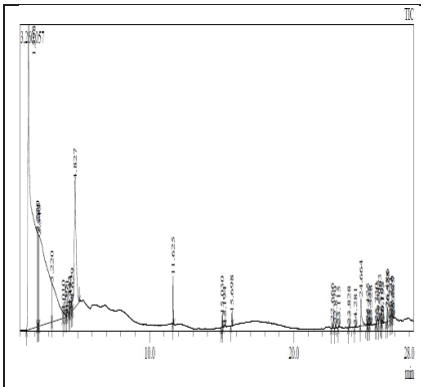


Exhibit-1

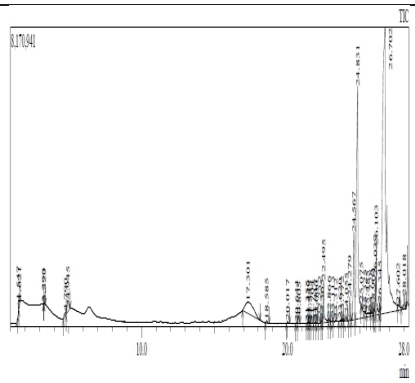


Exhibit-2

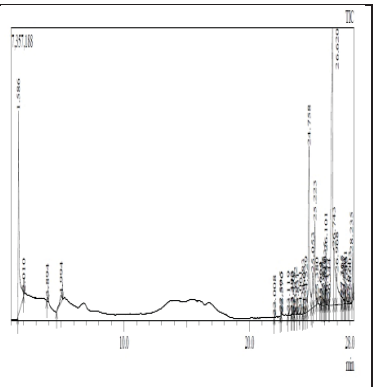
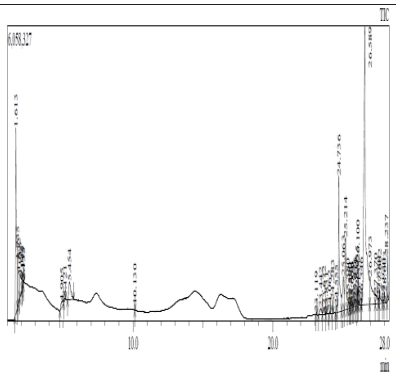


Exhibit-3



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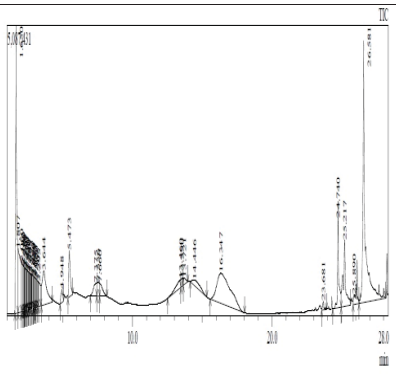
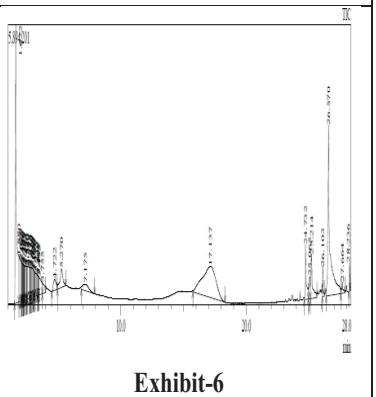


Exhibit-5



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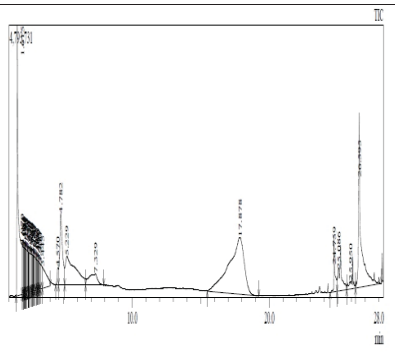

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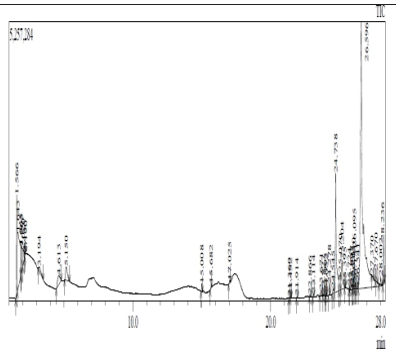

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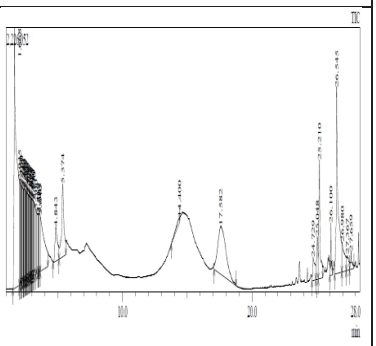

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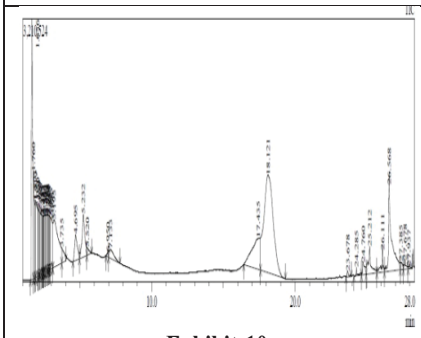

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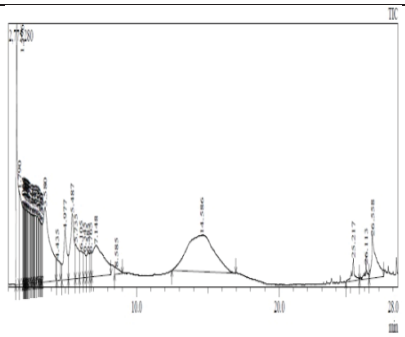

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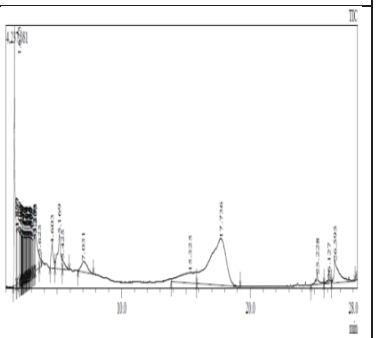

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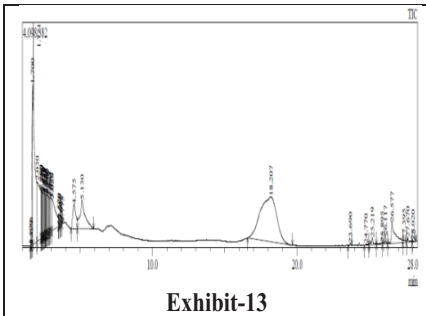

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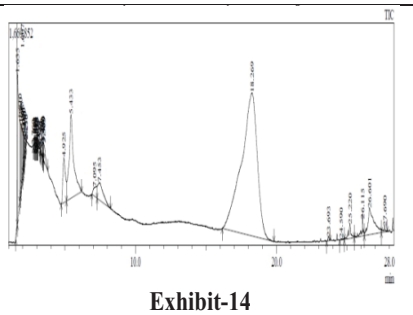

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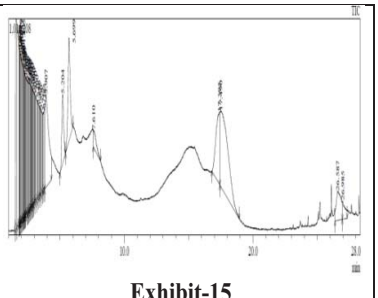

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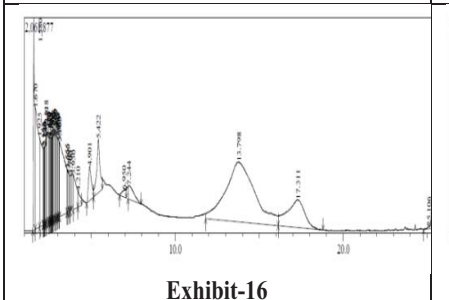

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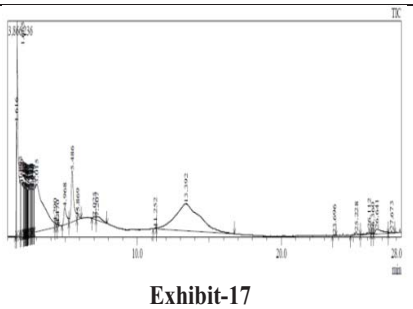

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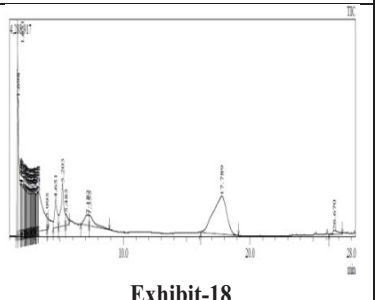

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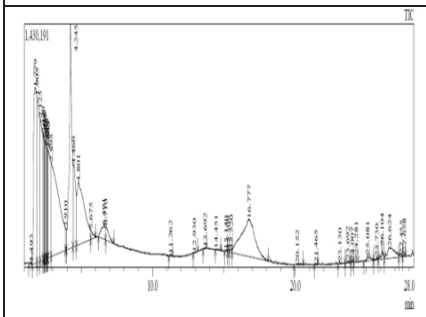

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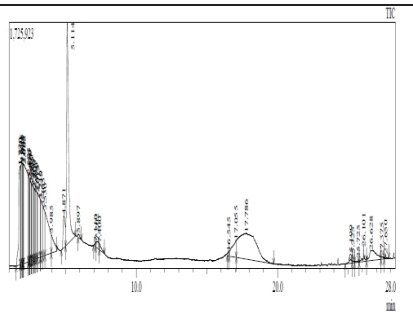

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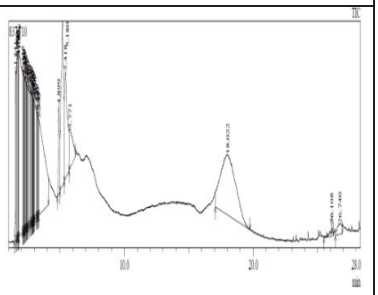

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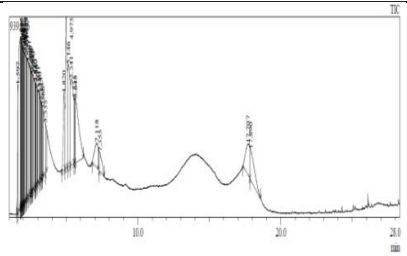

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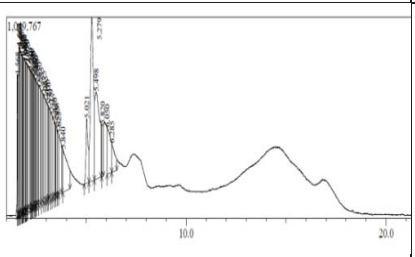

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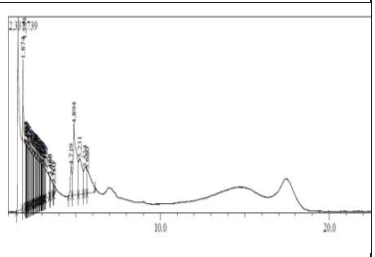

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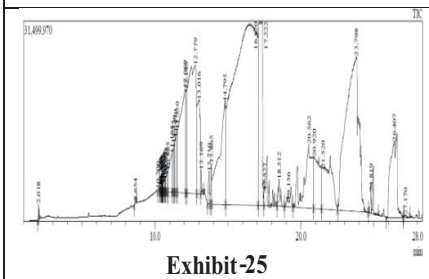

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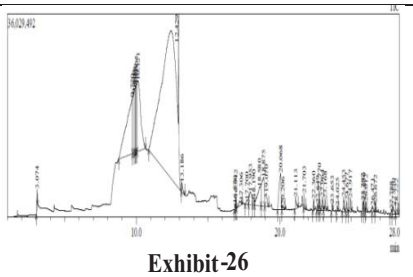

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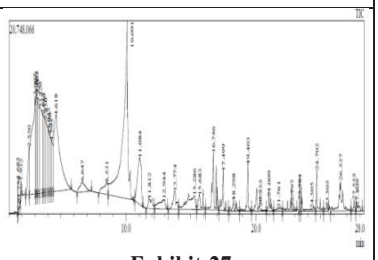

Exhibit-27

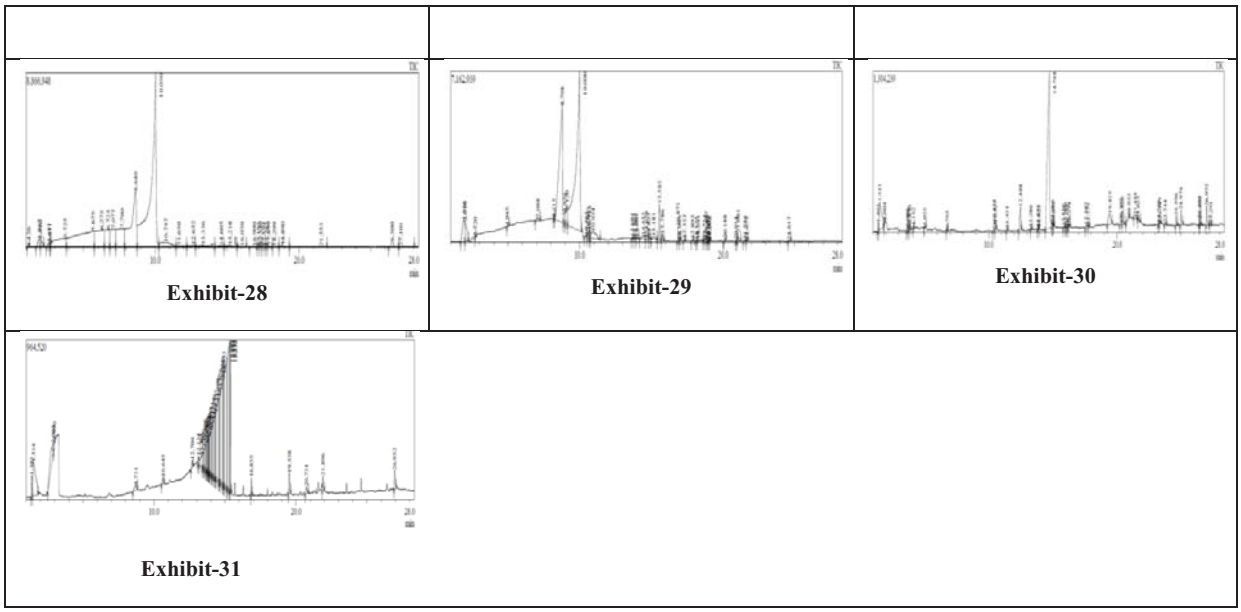

Fig. 3: Total Ion Chromatograms of Seized Samples (1- 31)

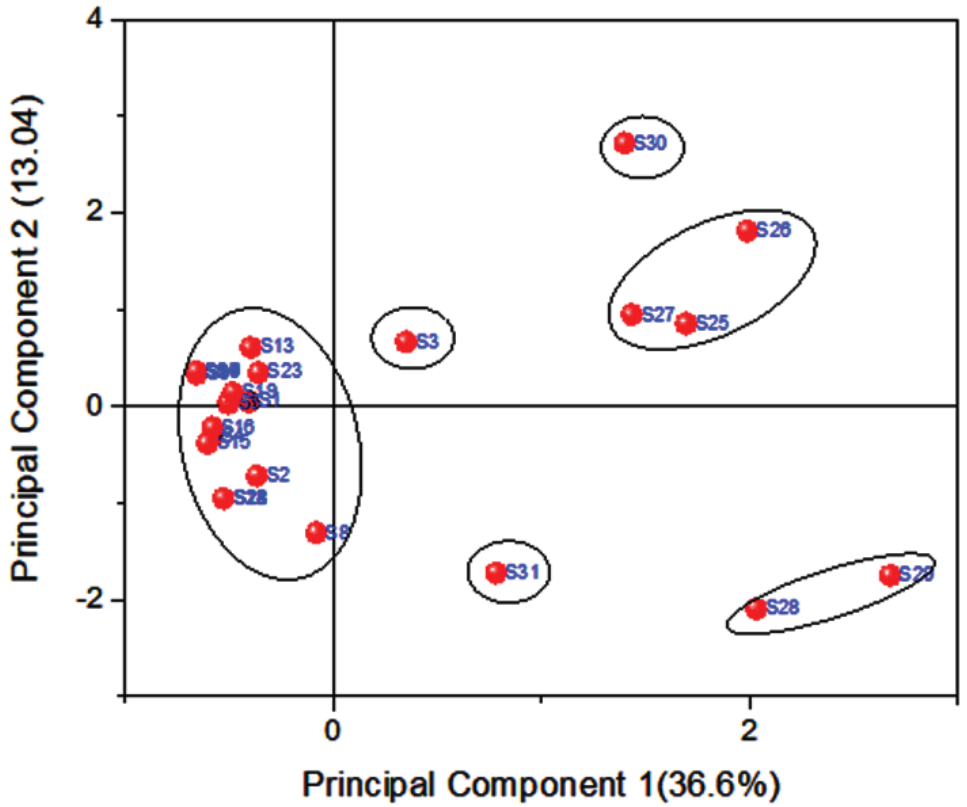

Fig. 4: Principal Component Analysis (PCA) using chemicals detected in seized moonshine's samples

One hundred eighty seven (187) chemical compounds were detected and identified by GC-MS technique in 31 moonshine's seized samples. These were categorised as Phytochemical compounds; Pyrolysis byproducts; Fermentation byproducts Pesticides; Organic solvent; Flavouring substances/ Essential oils and Antioxidant compound as depicted in table 2. The composition of these

samples were found quite different from recorded country liquor as the homemade producers might had use their own traditional methods.

Among these detected 187 chemical compounds, 73 chemical compounds were found health hazardous. The hazard statement of these 73 harmful chemicals are tabulated in table 3.

It is evident from table 3 that among 187 chemical compounds, 73 chemical compounds are

Table 3: Hazard identification of detected chemical compounds

S.No.	Chemical compounds	Detected in Exhibit No.	Hazards Identification								
			Irritant				Corrosive	Toxic	Flammable	carcinogenic	Toxic to aquatic Life
			Skin	Eyes	Respiratory	Digestive					
											
1	O-Ethylhydroxylamine	1, 4 to 7, 9 to 12, 14 to 22 & 24	✓	✓	✓	✓	-	✓	✓	✓	✓
2	Methanamine N-methoxy	1, 2, 8, 15, 20, 22 to 24 & 31	✓	✓	✓	✓	-	-	-	-	-
3 to 11	Methanamine, N-hydroxy-N-methyl/ N,N-Dimethylhydroxylamine	20, 22 & 23	✓	✓	-	-	-	-	-	-	-
	Dioxanediol/ 2,3-Dihydroxy-1,4-dioxane	1, 3 to 7, 9 to 22 & 24									
	Myristaldehyde	2									
	9-Tetradecenal	1 & 3									
	2-Decenal(E)-/ trans-2-Decenal	19									
	Pentadecanal	2, 8 & 20									
	Tetradecanoic acid	2									
	Pentadecanoic acid	2									
	n-Hexadecanoic acid	29 & 30									
12 to 14	Furfural	1 to 24 & 30	✓	✓	✓	✓	-	-	-	✓	-
	Furfuryl alcohol	30									
	BHA	29									
15	1,3-Dioxane, 2-methyl	25	✓	✓	✓	-	-	-	-	✓	-
16	Serinol/ aminoglycerin/ 2-amino-1,3-propanediol	23	✓	✓	✓	-	✓	-	-	-	-
17 to 31	Erythritol	1, 2 & 4 to 24	✓	✓	✓	✓	-	-	-	-	-
	Xylitol	7									
	Sorbitol	13									
	Ribitol	23									
	p-Ethylphenol	31									
	Methyl stearate/ Octadecanoic acid, methyl ester	2 to 4, 8 & 17									
	Glyceraldehyde	10, 15, 20, 22 to 24 & 30									
	Glycerin	1, 2, 4 to 24									
	Ethyl butanoate /Butanoic acid, ethyl ester	26 & 27									
	Isopentyl alcohol, acetate/ Isoamyl acetate	27									
	9-Octadecenoic acid methyl ester (E)/ Methyl elaidate	1 to 4, 6, 8 to 14, 17, 19 to 21									
	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)/ glycerine trioleate	4 & 5									

	Oleic acid, butyl ester	19									
	Isovaleraldehyde dibenzyl acetal	27									
	Benzaldehyde propylene glycol acetal/ 1,3-Dioxolane, 4-methyl-2-phenyl	27									
32	1-Butanol	27	✓	✓	✓	✓	-	-	✓	-	-
to	1-Pentanol/ n-Butylcarbinol/ amyl alcohol										
	Isoamyl alcohol										
36	Octadecanoic acid	1									
	Methyl Palmitoleate/ 9-Hexadecanoic acid, methyl ester (Z)	2, 3 & 8									
37t	Hexadecanal	1 to 4, 8 & 19	✓	✓	✓	-	-	-	-	-	-
o	9-Hexadecenal(Z)	2 & 28									
	Hexanophenone	2									
46	Elaidic acid/ 9-octadecenoic acid(E)	1 & 2									
	Butyl heptanoate/ Heptanoic acid, butyl ester	27									
	Octanoic acid, 3-methylbutyl ester/ Isoamyl caprylate	27									
	Dodecanoic acid, propyl ester/ Propyl decanoate	21									
	Formaldehyde dimethyl mercaptal S-oxide	1, 2, 4 to 24									
	Methyl tridecyl ketone /Pentadecan-2-one	2									
	1,3-Dioxane, 2-methyl	25									
47	Benzaldehyde, 4-chloro	1	✓	✓	✓	✓	-	-	-	-	✓
&	Dodecanoic acid	2									
48											
49	Dodecanal/ Lauraldehyde	28	✓	✓	-	-	-	-	-	-	✓
to	Acetoacetic acid, 3-thio-, benzyl ester/ Benzyl 3-thioxobutanoate	26									
51	Octanoic acid	29 to 31									
52	Acetic acid	1 & 23	✓	✓	✓	✓	✓	✓	✓	-	-
53	9-Hexadecenoic acid,(Z)/ Palmitoleic acid	2	✓	✓	✓	-	-	-	✓	-	-
& 54	Myristic acid, ethyl ester/ Ethyl myristate	27									
55	Ethyl heptanoate/ Heptanoic acid, ethyl ester	27	✓	✓	✓	✓	-	-	✓	✓	-
56	Propyl glycolate	3	✓	✓	-	✓	-	✓	-	-	-
& 57	9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester/ Glyceryl monooleate	3, 4, 5, 8, 10, 13, 14, 17 & 19									
58 to 60	Methyl tetradecanoate/ Myristic acid, methyl ester Tetradecanoic acid, methyl ester	2 & 3	✓	-	-	-	-	-	✓	-	-

	Isopentyl alcohol, propionate/ Isoamyl propionate	27									
	Oleic acid/ 9-Octadecenoic acid(Z)	30									
61	Palmitic acid, methyl ester/ Hexadecanoic acid, methyl ester	1 to 4, 8, 10 & 19	✓	-	-	-	-	-	-	-	-
62 & 63	13-Docosenoic acid, methyl ester/ methyl, 13-docosenoate	1, 3, 4, 7 & 8	-	-	-	-	-	-	✓	-	-
	Butyl ethanoate/ acetic acid, butyl ester	27									
64	9, 12-Octadecadienoic acid (Z,Z)-, methyl ester/ Methyl linoleate	2 to 4, 8, 9 & 15	✓	✓	✓	✓	-	-	✓	-	✓
65 & 66	Dodecanoic acid, methyl ester/ Metholene 2296	27	-	-	-	-	-	-	-	-	✓
	Acetic acid, phenylmethyl ester/ Benzyl ethanoate/ Benzyl acetate	19 & 27									
67	Ethyl caprylate/ Octanoic acid, ethyl ester	27	✓	✓	-	-	-	-	✓	-	-
68	Benzyl Benzoate	1	-	-	-	✓	-	-	-	-	✓
6 9	Formic acid, 2-methylpropyl ester	23	-	✓	✓	-	-	-	✓	-	-
70	Pentadecanoic acid, 3-methylbutyl ester/ Isopentyl decanoate	27	✓	-	✓	-	-	-	-	-	-
71	Isoamyl formate	27	-	✓	✓	-	-	-	-	-	-
72	Octadecanoic acid, 3-hydroxypropyl ester	26	-	✓	-	-	-	-	-	-	✓
73	delta.-Caprolactone	27	-	✓	-	-	-	-	-	-	-

found potential harmful to human health. These are categorized into six categories viz. Irritant, Corrosive, Toxic, Flammable, Carcinogenic & Toxic to aquatic life and among these 73 harmful chemical compounds, 40 chemical compounds are found only irritant, 2 are only flammable, 2 are only toxic to aquatic life; 13 are irritant & flammable; 7 are irritant & toxic to aquatic life; 4 are irritant & carcinogenic; 1 is irritant & corrosive; 2 are irritant & toxic; 1 is irritant, flammable & carcinogenic; 1 is irritant, corrosive, toxic & flammable and 1 is irritant, toxic, flammable, carcinogenic & toxic to aquatic life.

As per the table, it has also been observed that the most commonly present Irritant are O-Ethylhydroxylamine; Methanamine N-methoxy; Dioxanediol; Furfural; Glycerin; Methyl elaidate & Formaldehyde dimethyl mercaptal S-oxide; most commonly corrosives chemicals are Serinol & Acetic acid and the most commonly present toxic chemicals are O-Ethylhydroxylamine; Acetic acid & Glyceryl monooleate.

Further, it is also found that among 31 samples, sample marked as 1 to 25 and 27 and 30 are highly potential health hazardous samples as these samples are containing carcinogenic chemical compounds i.e. Furfural (in sample 1 to 24 & 30); Furfuryl alcohol (in sample 30); 1,3-Dioxane, 2-methyl (in sample 25); Ethyl heptanoate (in sample 27); BHA (in sample 29) & O-Ethylhydroxylamine (in sample 1, 4 to 7, 9 to 12, 14 to 22 & 24).

It is unequivocal that the coalesce presence of Methanol & carcinogenic chemicals (furfural, O-Ethylhydroxylamine) alongwith other harmful hazardous chemicals in sample 1 to 24 constitutes ***the most*** deadly chemical's combination drink and so, this make hooch lethal (toxic brew) to the vital organs.

Moreover, the presence of detected chemicals in myriad combination with statistical tools like principal component analysis as depicted in Fig. 4 are found to be advantageous in terms of interpreting the sources with adequate reliability. From these statistical analysis it is found that sample 1 to sample 24 (except sample 3) are from the same source; sample 25 to sample 27 are from same source; sample 28 & sample 29 are from same source; sample 30 & sample 31 are from different source.

CONCLUSION

Incidences of hooch tragedies have been increased in Punjab since 2020 and the main reasons observed for this tragedies are presence of hazardous

contaminants and additives besides methanol in hooch. The presence of these contaminants are mainly caused due to the ignorance of moonshiners towards public health in distillation process such as use of heads & tails in hooch; not using multiple distillation process; utilizing large containers of hazardous chemicals/organic solvents/pesticides; improper cleaning of raw materials for production of liquor and lack of facility to control the fermentation which causes oxidation of the ethyl alcohol into acetic acid. Moreover, to make brew strong and aromatic, moonshiners also add multiple harmful flavouring substances. The intake of these hazardous additives and contaminants in hooch can cause severe health problems viz. eye, nose & throat irritation; inflammation to monocytes; birth defects; allergic reactions; effect on reproduction, immune or nervous system; cancer & even death. The aforementioned health hazards in lieu of hazardous additives and contaminants besides methanol need utmost attention and stringent action by Govt. authorities/ law agencies for determining the cause of death/grievous hurt and in deciding the quantum of punishment. Further, the excise department should disposed off seized liquor laced with hazardous chemicals appropriately to protect other life as "what kills humans, can kill animal as well as aquatic life".

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Terrorism at Rise with the Chemicals Insight: Use of Chemical Warfare Agents an Issue of Global Concern

Neha Jain

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Abstract

Crime has led to a worldwide increase with a main weapon of offence including not only a physical object but show the incidences of involvement of chemicals also. Chemical warfare agents are one such example commonly employed by large group of people, mainly violent criminals who not only wants to create a terror or threat in the world but to cause war scale destruction. There are numerous of incidents reported from past showing the involvement of hazardous chemicals for committing crimes. Chemical Warfare Agents (CWA) are synthetic chemicals used in the warfare as weapons, which are highly toxic and lethal to the extent that can cause temporary incapacitation, permanent health damage and even death of the targets. Common examples of these agents are nerve agents, vesicants, incapacitating agents, blood agents, and riots control agents. These agents are variedly classified as per the above mentioned categories depending onto the effects and adverse effects they poses on human health and on society.

The rate of crime commission using these hazardous agents is very rapid, thus making it an issue of serious concern to take the measures to prevent the innocent individuals.

Keywords: Chemicals; Chemical Warfare Agents; Destruction; Hazardous; Weapons.

INTRODUCTION

Crime has increased tremendously in the world day by day, with the major target either being a single individual or sometimes the entire population. The usual trends seen in the crime are generally murder, assaults, burglary, etc. committed using any particular weapon of offence but when the crime has occurred at a global level it targets the entire country. Such crimes has been put forth via chemicals commonly known as chemical warfare agents to cause devastation at a large scale. For example, the bombings occurred at the World Trade Centre, Oklahoma City's Federal Building, Nerve gas attacks in Japan etc. reveals the attacks of terrorism in different parts of the world. The history is full of such terrorist attacks (anthrax attack etc.), taken place by the widespread use of extremely hazardous chemicals termed as chemical warfare agents. The threat of chemical weapons has spread from the battlefield to cities and towns due to the threat of international terrorism.[1]

The FBI has defined terrorism as the unlawful

Author Affiliation: [1]Former Senior Scientific Officer, Sherlock Institute of Forensic Science India, GTB Nagar, Kingsway Camp, Hudson Lane, Delhi 110009, India.

Corresponding Author: Neha Jain, Former Senior Scientific Officer, Sherlock Institute of Forensic Science India, GTB Nagar, Kingsway Camp, Hudson Lane, Delhi 110009, India.

E-mail: neha.jain258992@gmail.com

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use of force or violence against persons or property to intimidate or coerce a government, civilian population, or any segment thereof, to furtherance of political or social objectives (FBI, 2003). The first large scale chemical terrorism incident occurred in the United States in 1982 (Cooke, 2002). Seven relatively young people in the Chicago, Illinois area collapsed suddenly and died after taking Tylenol capsules that had been laced with 65–100 mg cyanide per capsule.[7]

Chemical Warfare Agents (CWA) are synthetic chemicals used in the warfare as weapons, which are highly toxic and lethal to the extent that can cause temporary incapacitation, permanent health damage and even death of the targets. These are fast acting substances generally classified based on the harassing, incapacitating and lethal effects produced. Nowadays, the chemicals most commonly used for causing destruction at a large scale involves chlorine, mustard or nerve agents because of their invisible property, extreme potent and high toxic nature.[5]

These agents were synthesized for the first time during First World War, with the use of chlorine gas. Later, the discovery occurred at a fast pace that a variety of other gases such as sulphur, mustard etc. were also used for causing mass level destruction. During 1930's various military forces across the world were actively synthesizing Organophosphorus pesticides that can be used as nerve agents.[7]

The use of these CWAs is not a new thing as various incidents from the history reported (Iran-Iraq War in the 1980's, 1995 Tokyo subway attack) the attacks and casualties resulting from therein due to the use of these hazardous chemicals. The current conflicts between in Syria is also deemed to have seen the use of these chemicals in a number of incidents that have resulted in numerous deaths and long term health effects among the victims.[3]

There has been numerous of incidences indicating the use of chemical warfare agents not only in India but across the globe. For instance, Kim Jong Nam, a native of North Korea was killed in Februaryby smearing the nerve agent VX on his face at Kuala Lumpur airport terminal, Malaysia. Similarly another incident witnessed the death and disfig. ment of several individuals including children in Khan Sheikhoun town in the North-western Idlib Governorate in Syria by the attack of nerve agent in April which is similar to the chemical attack occurred in August 2013 in Ghouta that killed over a hundred people near Damascus, Syria's capital.

Another incident reported the death of former Russian spy and his daughter due to poisoning with the extremely hazardous nerve agent in 2018 at south west England, near the town of Salisbury in Amesbury. On July 8, 2018, a 44-year-old British woman was also died due to exposure to a highly toxic nerve agent in extremely high amount at the same place.[5,6]

In asymmetric warfare and terrorism, it is sometimes difficult to recognize or identify the enemy. Because terrorists may avail themselves of toxic industrial chemicals and materials that are transported and already stockpiled, a working knowledge of the chemistry of chemical warfare agents is no longer a necessity. It is important to recognize that the advances in biotechnology, nano technology, genetic engineering, neurobiology, computer sciences among others, may assist not only in the proliferation of traditional chemical warfare agents, but also stimulate the emergence of non-traditional agents as well. Advances have also occurred in the delivery systems of these agents.[8]

The rise of terrorism and the conditions of war has led to a great increase in the production of these harmful disastrous chemical weapons. For instance, the present day situation of War between India and China, China and Tanzania and many other countries may increase the chances of employing these harmful chemicals which leads to large scale destruction. Not only this the use of these harmful chemicals is becoming so prevalent in every war situation by many countries that in India recommendation are made by parliamentarians that "all kinds of war in future may be fought using NBC (Nuclear, Biological and Chemical) weapon systems" and an adequate budgetary provision needs to be earmarked for Research and Development efforts of the DRDO or the Defence Research and Development Organization in the field of NBC warfare for thereof.[4]

TOXIC PROPERTIES

The susceptibility to the effects of these chemicals depends mainly on the dose and time of exposure and individual health circumstances. These CWAs exist mostly in the form of liquids or solid particles and usually disseminated in the environment as aerosols so as to make them spread in the form of a pure substance in solid or liquid form consisting of colloidal solid or liquid particles suspended in water or other solvents. These aerosol particles remains in the air for an indefinite period of time

and thereby make entry into the body of the target.[8]

TYPES OF CHEMICAL WARFARE AGENTS

These are of majorly five types

a. ***Riot-Control Agents (RCAs):*** These are the chemicals that rapidly cause irritation on exposure of all oronasal and conjunctiva mucosal tissues and respiratory tract. These agents typically cause disabling effects, temporary pain and discomfort. Examples Pepper spray and tear gas are the well known riot control agents used by military and law enforcement personnel. The active chemical present in pepper spray consists of oleoresin capsicum (OC), a mixture of naturally occurring substances extracted from capsicum plants like chili peppers, cayenne pepper, red peppers and jalapenos containing the main irritant capsaicin which is a colourless solid irritant. Similarly tear gas consists of ortho-chlorobenzylidene-malononitrile (CS), as the main component specifically affects the peripheral and sensory nerve endings of the mucous membranes and skin, causing irritation and intense pain. Irritation and burning sensation in the nose and mouth followed by salivation and excessive nasal discharge are also among the most common symptoms. Maximum effects occur within 20–60 seconds after exposure and can persist for 5–10 minutes.

b. ***Incapacitating agents:*** These are themind-altering chemicals also referred to as 'psychochemicals', causing mental disability, disorientation and make the target incapable of performing normal functioning. Dizziness, giddiness, sedation, respiratory depression, restlessness, mental confusion etc. are the most common symptoms appeared. The symptoms last from few hours to few days. Common example includes Fentanyl, lysergic acid diethylamide (LSD-25), and 3-quinuclidinyl benzilate (BZ).

c. ***Blood agents:*** Blood agents are the most toxic gaseous chemicals containing cyanide as the main component which gets readily absorbed into the bloodstream via inhalation. These agents interfere with cellular respiration, and block the uptake of oxygen. This oxygen stoppage will make the cyanide compounds to proliferate rapidly and causing the body to suffocate and asphyxiate. Common examples of blood agents are hydrogen cyanide (HCN) and cyanogen chloride (ClCN). The exposure time is almost 30 seconds resulting in loss of consciousness apnoea within 3–5 minutes, violent seizures and cessation in cardiac activity due to loss in respiration control. The continuous exposure for about 5 to 8 minutes will eventually leads to death.

d. ***Vesicants:*** Vesicants are the chemicals also known as blister agents because of their ability to affect skin and tissues and thereby causing burns or blisters upon contact. These agents are not usually lethal until exposure occurs in higher doses, although a piercing pain is felt immediately after the contact. Common examples are mustards, lewisite and phosgene oxime etc. Out of these mustard is considered to be highly toxic and extremely poisonous with an LD50 of 7g/person to the skin, and exposure to as little as one gram via inhalation can cause death within 30 minutes.

e. ***Choking Agents:*** These are other weapons of this category also referred to as respiratory agents, due to their ability to target mainly the respiratory tract, specifically nose, throat, and the lungs. These agents have the tendency to cause damage to the membranes between the air sac of lungs upon inhalation and thereby causing difficulty in breathing and ultimately lung damage. Due to the effects of these lethal chemicals the lung membranes gets filled with the fluid, leading to pulmonary edema and respiratory failure. Common examples of choking agents include phosgene ($COCl_2$), diphosgene, chlorine (Cl_2) and chloropicrin ($-CCl_3NO_2$)

f. ***Nerve Agents:*** These are the most widely used chemicals for destruction purposes. These are chemically known as organophosphoric acid esters or organophosphorous compounds, which inhibit the activity of the enzyme responsible for normal muscle and glandular function i.e. acetylcholinesterase. The major effects will be on skeletal muscles, certain organs, and the central nervous system. These compounds are similar to, but much more deadly than, agricultural organophosphate pesticides. These nerve agents halts the functions of this acetylcholinesterase, resulting in an accumulation of acetylcholine at the nerve endings which will affect the nerve impulses from the nervous system resulting in an involuntary and uncoordinated muscle movements. Although Nerve Agents are hazardous through inhalation, skin and eye exposure, ingestion, and abraded skin (e.g.,

breaks in the skin or penetration of skin by debris).

Nerve agents are broadly classified into two types:

- ***G Series:*** are organophosphate esters containing fluorine or cyanide compounds first manufactured by Germans as insecticides. These chemicals sooner recognised as potential chemical warfare agents with a 'faintly fruity' or 'spicy odour. In 1936 the first nerve agent i.e. 'Tabun' was synthesised. This discovery was followed by Sarin in 1939 and Soman in 1944, the other well known and highly potent nerve agents. The lesser known Cyclosarin (GF) was discovered in 1949. These agents have lethal concentration of 1 ppm over 10 min of exposure. Common examples of well known nerve agents are Tabun (GA), Sarin (GB), Soman (GD), and Cyclosarin (GF). "G" series Nerve Agents are hazardous through inhalation, skin and eye exposure, ingestion, and abraded skin (e.g., breaks in the skin or penetration of skin by debris).[3,5,7]

General Chemical Structure

R-P(O)(X)-OR' or R2N-P(O)(CN)-OR' or R-P(S)(X)-OR'

- ***V series***: are the compounds which contain sulphur as their main ingredient. These agents were first synthesized during 1950s in United Kingdom by the Scientists working on the esters of Organophosphorus pesticides group. These agents have a low volatility and high persistence hence remain on clothes and other surfaces for a long time after application. These are of different types-VX (Oethyl–S–[2(diisopropylamino) ethyl] methylphosphonothioate), VE (O-ethyl-S-(2-diethylaminoethyl-) ethyl-phosphonotioate), VG (O,Odiethyl-S-(2-diethylaminoethyl)-phosphorotiate), VM (O-Ethyl S-(2- (diethylamino) ethyl)methylphosphorotioate) and VR S-[2-(diethylamino)ethyl] O hydrogen methylphosphonothioate. Out of these VX is considered as the most lethal and toxic agent compared to the others and is hard to detect physically due to its odourless and tasteless properties and their percutaneous exposure. These V-agents series are more potent as than G-series due to their higher stability, greater resistance to detoxification and ability to easily penetrate skin.[3,5,7]

General Chemical Structure

R-P(O)(OR')-$SCH_2CH_2NR_2$

Suggestive Measures to Counteract

The rate of crime commission using these hazardous agents is very rapid, thus making it an issue of serious concern to take the measures to prevent the innocent individuals. Detection of hazardous chemicals and now chemical weapons is a requirement for first responders of all sorts.

During the 88th session of the Executive Council of the Organization for Prevention of Chemical Weapons (OPCW), Ambassador and Permanent Representative of India to OPCW, Venu Rajamony stated that the use of these hazardous and toxic chemicals is contrary to the provisions made by Chemical Weapons Convention (CWC) and their use is violating the set legal norms. These weapons shows a complete disregard of humanity and a total threat to human population therefore, considered as a matter of great concern and hence, required implementation of effective methods and measures which eliminate all possibilities of any future use of chemical weapons and uphold the global norm against use of chemical weapons. Policies must be made so that if these are released the entire world will not be effected.[6]

CONCLUSION

The threat of use of these hazardous chemicals during the war is considered to be a global matter of concern as these substances not only poses harm to humanity and human beings but they have the potential to destroy entire world. Therefore the release of these dangerous weapons in the environment should be monitored at a regular pace by designing and implementing strict legal policies and norms.

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Case Report

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Reperfusion Induced Fatal Hemorrhagic Myocardial Infarction: A Case Report

Gokul G[1], Abdul Raoof MP[2], Abhishek Yadav[3], Amar Ranjan[4], Manivel. S[5]

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Abstract

Sudden death due to coronary artery disease is the leading cause of death globally. Occlusive coronary artery disease results in ischemia of myocardial tissue and subsequent infarction. Various treatment modalities like blood thinners, percutaneous coronary intervention (PCI) etc are being employed in the treatment of acute myocardial infarction (MI). However, these modalities have their own limitations. In this case, reperfusion therapy resulted in hemorrhagic myocardial infarction (HMI) with a fatal outcome. An adult male with history of sudden collapse was brought to emergency department, where he was declared as brought dead. He underwent reperfusion therapy and was on blood thinners, 4 days before the incident. On autopsy, we could find the features of hemorrhagic myocardial infarction grossly and the same was confirmed histopathologically. Though reperfusion injuries are commonly documented, deaths due to hemorrhagic myocardial Infarction, which is one of the rare complications of reperfusion therapy, are rarely reported. The important role of forensic pathologists in this case is to identify the rare causes, which led the patient to death during management of occlusive coronary artery disease.

Keywords: Occlusive coronary artery disease; Reperfusion therapy; Hemorrhagic Myocardial infarction; sudden death.

INTRODUCTION

Coronary artery disease is the leading cause of death worldwide.[1] Few reasons include urbanization, sedentary lifestyle, prevalence of alcohol and smoking in the developed societies.[2] After an acute myocardial ischemic episode, timely and efficient myocardial reperfusion with the use of primary percutaneous coronary intervention (PCI) or thrombolytic therapy is the most effective method for reducing the size of infarct and improving the outcome.

The restoration of blood flow to the ischemic myocardium may paradoxically reduce the beneficial effect and can lead to lethal reperfusion injury in some cases. It is defined as myocardial injury caused by the restoration of coronary blood flow after an ischemic episode. The injury culminates in the necrosis of cardiac myocytes that were viable immediately before reperfusion.[3]

Author Affiliation: [1,5]Junior Resident, [2]Senior Resident, [3]Additional Professor, [4]Additional Professor, Institute Rotary Cancer Hospital, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

Corresponding Author: Abhishek Yadav, Additional Professor, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

E-mail: drayad_in@yahoo.com

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This form of myocardial injury, which induces death of cardiomyocyte and increases infarct size, may in part explain why, despite optimal myocardial reperfusion, the death after an acute myocardial infarction is not rare.[4]

The progression and presentation of ischemic heart disease is highly variable, ranging from silent MI to spontaneous cardiac rupture. Hemorrhagic Myocardial Infarction is one such potentially life-threatening complication of coronary revascularization following acute myocardial infarction. The authors report here a case of hemorrhagic myocardial infarction in an adult male diagnosed at autopsy and discuss its significance and implications in clinical outcome of such patients.

CASE HISTORY

A 40-year-old Japanese male was admitted with complaints of abdominal pain and vomiting to a super specialty hospital in New Delhi, India. He was managed initially under gastroenterology department, where symptomatic treatment was given. Due to persistent pain, multiple investigations including cardiac markers have been ordered. The serum CK-MB level was 183.8 ng/ml (Normal range: 0.6-6.3) and Trop-I was 22.08 ng/ml (Normal range:<0.02). In view of elevated cardiac markers, coronary angiography was ordered which revealed critical single vessel disease. Subsequently, the patient underwent PTCA (percutaneous transluminal coronary angioplasty) with stent (Xience XP Edition which was approved in Japan, introduced by Abbott) to Left anterior descending artery (LAD) under OCT (Optical coherence tomography) guidance on emergency basis with an uneventful course. The patient was discharged in stable condition after 3 days and was prescribed antiplatelet drugs (ecospirin, ticagrelor), statins (rosuvastatin), beta blocker (metoprolol), ACE-inhibitor (ramipril), anti-anginal drug (trimetazidine) and antibiotic (cefuroxime). Approximately 9 hours had already been passed before cardiac markers were taken since the arrival of patient to the hospital. By the time, stent was placed in the proximal segment of left anterior descending artery (LAD), it took further one hour.

In the following day, at the Police Control Room (PCR), a call was received regarding an adult male, found lying unresponsive in prone position at his residence. He was taken to a nearby hospital where he was declared as brought dead. His body was then sent for postmortem examination.

AUTOPSY FINDINGS

Rigor mortis was present over all parts of the body. Postmortem lividity was present on the right side of face, dependents parts of the body in prone position with contact pallor, which was not fixed. Conjunctivae were congested. No external injury is present over the body.

Pericardial sac was intact. Pericardial cavity contained about 40 ml of straw colored fluid. The anterior free wall of the left ventricle had patchy areas of dark reddish discoloration. The infarct zone was transmural and was extending till the endocardium (Fig. 1a, 1b).

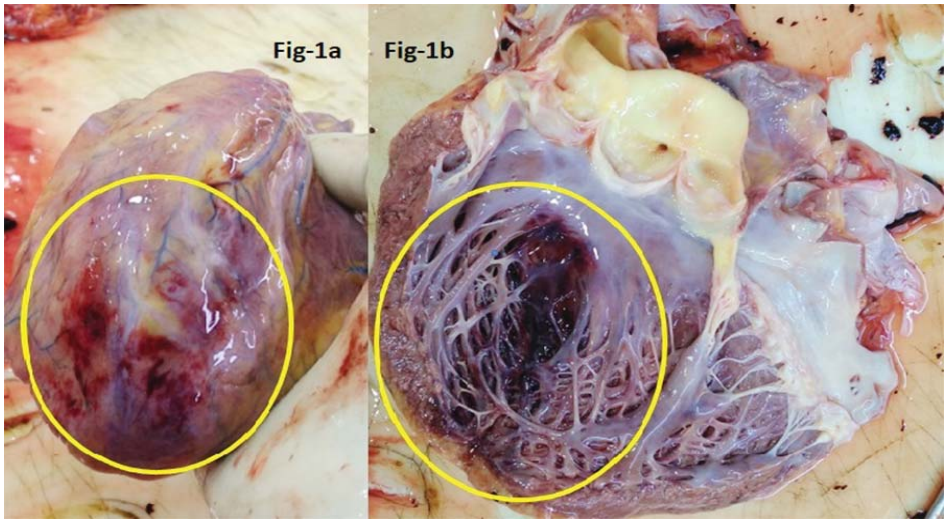

Fig. 1a: Reddish infarct on the anterior surface, **Fig. 1b:** Dark reddish infarct extending into the endocardium of LV

Left anterior descending artery had a metallic stent of length 4.8 cm along the proximal and middle one third of its course (Fig. 2). Left circumflex and right coronary artery were patent. The thickness of the left ventricle was within normal limits. Lungs were congested and edematous weighing 720 gm and 690 gm. Brain and liver were congested. All other internal organs were grossly unremarkable.

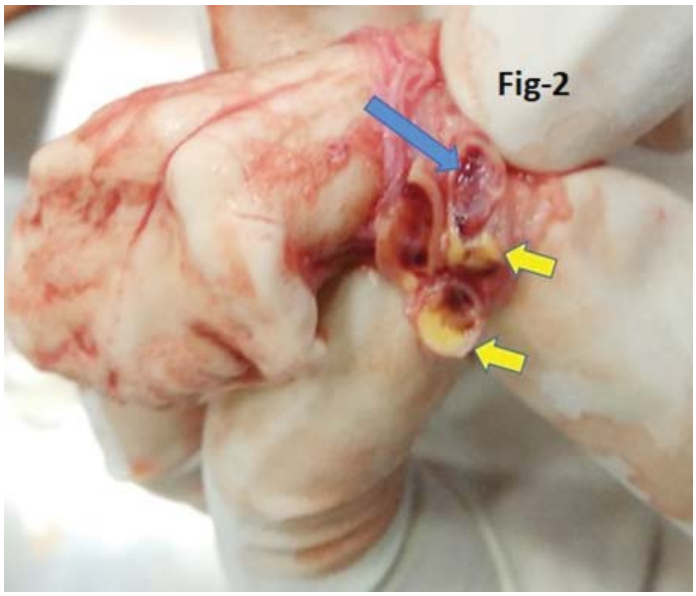

Fig. 2: Atherosclerosis in LAD (yellow arrows) with stent in-situ (Blue arrow)

HISTOPATHOLOGICAL EXAMINATION

Histopathological examination of the heart was performed. Patchy areas of necrotic muscle fibers are present in the anterior wall of left ventricle (Fig. 3a, 3b, 3c). In addition, extensive intramuscular hemorrhages are present in the anterior free wall of left ventricle. The lumen of the left anterior descending artery showed critical narrowing resulting in about 50-60 & occlusion of lumen due to atherosclerotic plaques, which are adherent to the vessel wall.

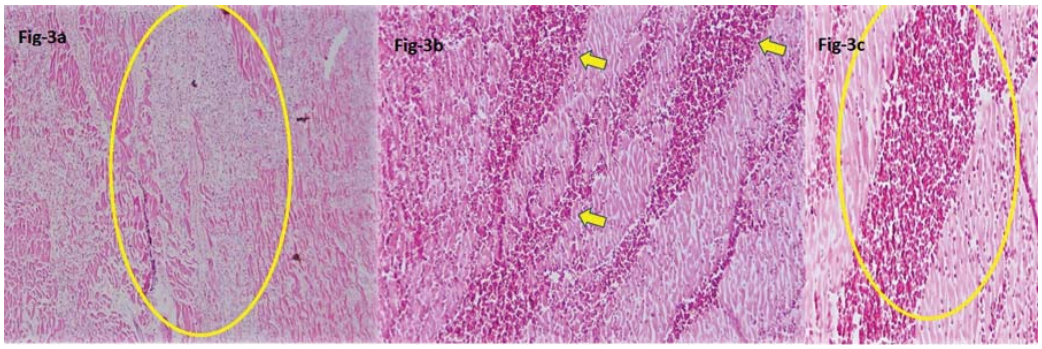

Fig. 3a: Pale areas of myocyte necrosis (Light microscopy-4X)
Fig. 3b: Intramyocardial hemorrhages (Light microscopy-10X)
Fig. 3c: Intramyocardial hemorrhages (Light microscopy-40X)

DISCUSSION

The aim of reporting this case is to discuss importance of acute hemorrhagic myocardial infarction (HMI) after re-perfusion therapy and morphological and histological findings of hemorrhagic myocardial infarction. Solving cases of sudden deaths are a daily challenge for the forensic pathologist. Acute coronary syndromes form a bigger part of the spectrum of sudden deaths and its incidence is more common in men than in women.[5] Myocardial infarction (MI) is becoming more frequent in the younger ages in the recent times.[2]

Infarction can be hemorrhagic or anemic (ischemic).[6] Myocardial infarction commonly

involves ischemic injury of the myocardium, which occurs when there is a negative balance in the perfusion and myocardial demand. The ischemic area undergoes metamorphosis subsequently depending on the amount of perfusion, type of myocardial resuscitation, the time taken for intervention and the preexisting hypertrophy of myocardium (most sensitive factor for necrosis).[7] The adverse effects of reperfusion are more evident when duration of ischemia crosses the golden period of 3 to 4 hours.[7,8,9] (As they say, Time is Myocardium). The infarct size is a directly related to severity of arrhythmia and mortality.[10]

In accordance with WHO, India ranks fifth among such deaths in the younger population.[11] Advancing research provides the healthcare personnel with various tools to be used in the management of Acute MI. This could be pharmacological thrombolysis or percutaneous coronary intervention. Reperfusion is a panacea in cases of acute myocardial infarction. Nevertheless, contradicting evidences are being stated by researchers for each technique, these modalities are virtually applied in almost all cases. Though the thrombolytic methods enable the clinician to establish reperfusion, the extent of reperfusion injuries and its adverse effects are not uncommon. Many theories of reperfusion like oxygen based free radicle theory, calcium theory, the PH theory, inflammatory response are proposed which in virtue of their own mechanism, cause extensive myocardial damage.[1] However, whether reperfusion by itself causes injury or it hastens the process of necrosis in irreversibly damaged myocardium is widely debated.

Mechanism of (Hemorrhagic Myocardial Infarction) HMI: When acute compromise to coronary blood flow occurs, the vascular endothelium in addition to the myocardium also undergoes ischemic damage. When perfusion is reestablished, the areas of endothelial damage become a potential site of hemorrhage into the surrounding myocardium. This is amplified by the therapeutic anticoagulation provided for reperfusion, increasing the probability of intramyocardial hemorrhage. The resulting hemorrhage into the myocardium increases the interstitial pressure, which has been already caused by tissue edema. This result in compromise of coronary blood flow in the area of at-risk-myocardium. These series of events cause a progressive wavefront like mechanism of myocardial injury in a patient with acute reperfusion.[9]

On gross appearance, the ischemic myocardial tissue shows soft, friable and tigroid appearance, as they evolve where as the hemorrhagic myocardial tissue is hard and dark red. Based on gross appearance, HMI can be classified as massive (gross + microscopic visibility) or focal (only microscopic visibility).[12] It can be graded as mild, moderate or severe based on the area of involvement.[13]

Histopathological examination of the area of interest shows accumulation of erythrocytes between the myocytes associated with areas of myocardial necrosis. Myocyte necrosis is not always mandatory since a remote infarct can cause hemorrhage in the adjacent myocardium.[14] Microscopically, it will appear as hemorrhage amidst between normal myocardial fibres.[13] This, in turn can potentially cause microinfarcts in the vicinity. In addition, the necrotic myocardial tissue, usually clearly by phagocytosis in ischemic infarcts are relatively dormant in hemorrhagic infarcts.[14] In cases of sudden death due to ischemic MI, due to lack of survival time and perfusion, gross pathological findings could not be appreciated at autopsy. However, in cases of hemorrhagic myocardial infarction cases, the survival time with reperfusion facilitates the necrosis of myocytes and the findings are appreciated grossly during autopsy.

HMI is a serious complication of reperfusion of acute MI and the diagnosis of which could be made by T2 weighted cardiac MRI.[15,16] The hemorrhagic conversion of ischemic area occurs approximately in one third of patients with STEMI and PCI.[17] In our case, almost 10 hours have been passed before the onset of reperfusion. Appropriate and timely management of patient with blood thinners and PCI will provide better prognosis.

CONCLUSION

The process of myocardial reperfusion itself, may induce injury to the myocardium, thereby reducing the beneficial effects of myocardial reperfusion even after the patient is discharged. HMI is underreported in underdeveloped and developing countries, due to limited facilities especially MRI. The forensic pathologists should not only restrict their expertise to unnatural deaths, but also explore the various causes and presentation of natural deaths with detailed histopathological examination in all cases of death due to acute coronary syndromes with reperfusion therapy. The role of forensic pathologist serves a significant part in the evolutionary development of evidence based medicine and new cardioprotective strategies

for the benefit of patients with acute myocardial infarction and toavoid myocardial reperfusion injury.

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Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

Tables and fig.s

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- fig.s necessary and of good quality (color)
- Table and fig. numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
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- Credit note for borrowed fig.s/tables provided
- Manuscript provided on a CDROM (with double spacing)

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