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Contents

Original Articles

Development of New Solvent Systems for the Analysis of Triazophos Pesticide Extracted from Blood 5
Nand Lal, Ashok Kumar Jaiswal, Adarsh Kumar, Madhuri Gupta

Effects of Temperature & Putrefaction on the Analysis of Carbofuran & Carbaryl Insecticides 11
P. Sharma, Risha Jasmine Nathan Singh

Detection and Identification of Chlorophenraminemaleate in Street Narcotic Stuff 15
Vinod Dhingra

Review Articles

Screening/Spot/Colour Test of Anti-Depressants 17
A.K. Jaiswal, Kamna Sharma, Sally Lukose, T. Millo, Anu Singla, Kulbhushan Prasad, S.K. Gupta

Phenol Poisoning with Analytical Aspects and Its Management 25
Mohit Gupta, A.K. Jaiswal, Abhishek Yadav, S.K. Gupta

Forensic and Pharmacognostic Studies of Jatropha Curcas 31
Baljeet Yadav, Anu Singla, Vinod Dhingra

Doctor's Witness and Court Procedures in India 37
Asit Kumar Sikary, Millo Tabin, D.N. Bhardwaj

Guidelines for Authors 45

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Original Article

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Development of New Solvent Systems for the Analysis of Triazophos Pesticide Extracted from Blood

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Abstract

Triazophos is considered as Organophosphorous poison. Although several instrumental methods like UV-VIS, GC, and HPLC are available for separation and identification of Triazophos, but cost of analysis is very high. Therefore a simple, cheap, rapid and reliable Thin Layer Chromatography (TLC) method for separation of Triazophos has been presented. Triazophos was first extracted from blood and then identified on TLC plates by using various solvent systems. The detection of spots on the developed TLC plates was performed by using Bromophenol Blue followed by 4% Acetic acid as the spraying reagents. There were 9 different solvent systems with different volumetric ratios used in separation and identification of Triazophos.

Keywords: Triazophos; Organophosphorous; Solvent Systems; TLC; R_f; Spraying Reagents.

Introduction

Triazophos a contact and stomach poison for insects and mites. It controls a large number of insect, pest and mites that damage agricultural, horticultural and forest crops. It is mainly used in the field of cotton, sugar cane, maize, potatoes, vegetables, fruits, coffee and ornamentals [1]. Its IUPAC name is *O,O*-diethyl *O*-1-phenyl-1*H*-1,2,4-triazol-3-yl phosphorothioate. The molecular formula of the compound is $C_{12}H_{16}N_3O_3PS$ with the exact molecular mass of 313.3 g/mol [2]. The structural formula of Triazophos is depicted in Figure 1.

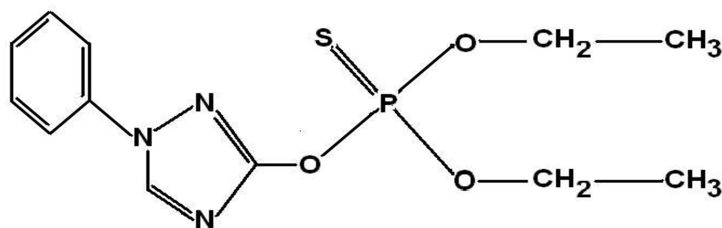

Fig. 1: Chemical structure of trizophos

It is an oily liquid having a yellowish appearance. It has a melting point of 2-5°C. The solubility of the compound is 30-40mg/kg in water, more than 500 g/L in acetone, 2-Propanol, ethyl acetate, and methanol and in Dichloromethane. Acute oral LD50 is administered to rats the dosage being 82 mg/kg and for dogs 320 mg/kg. Acute percutaneous LD_{50} is administered to rats in amount 1100mg/kg.

Reactivity Profile

Organophosphates, such as Triazophos, are

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susceptible to formation of highly toxic and flammable phosphine gas in the presence of strong reducing agents such as hydrides. Partial oxidation by oxidizing agents may result in the release of toxic phosphorus oxides.

Health Hazard

Triazophos is a cholinesterase inhibitor and acts on the central nervous system. Organic phosphorus insecticides are absorbed by the skin as well as by the respiratory and gastrointestinal tracts.

Fire Hazard

Some of these materials may burn but none of them ignite readily. Container may explode in heat of fire. Fire may produce irritating or poisonous gases. Degraded by acids and alkalis.

The various techniques such as high performance liquid chromatography [HPLC][3-5], gas liquid chromatography [GLC][6], Fourier transform *infrared* spectroscopy[7], high performance thin layer chromatography [HPTLC][8,9] etc. have been used for the analysis of various organophosphorous pesticides. In present study an attempt has been made to analyse Triazophos from blood by *Thin Layer Chromatography* [TLC]. The advantages of TLC are the low cost simultaneous analysis of large number of samples and minimum sample preparation[10-11].

Experimental

Materials and Reagents

Silica gel G (Glaxo India Ltd., Mumbai), Acetone (Merck Specialties Pvt. Ltd.), Ethylacetate (Glaxo Smithkline Pharmaceutical Ltd., Mumbai), Dichloromethane, n-Hexane, Conc. Sulphuric acid, 2-Propanol, Benzene, Petroleum benzene (E. Merck India Ltd.) Chloroform, Ethanol (Merck Ltd., Mumbai).

Beaker, conical flask. TLC glass plates, glass rod, glass chromatographic chamber, separating funnel, volumetric flask and fine capillary tubes.

Preparation of Standard Solution

The 1000 ppm solution of Triazophos was prepared by dissolving 0.1 gm of Triazophos in 100 ml of n-Hexane.

Preparation of Spraying Reagent

1. 4% Acetic acid: Measure 4 ml of acetic acid and then make its volume up to 100 ml by adding 96 ml water.
2. Bromophenol blue:

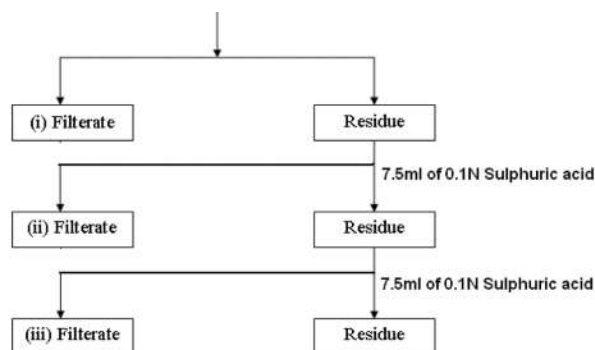
Solution 1 : 0.25gms of Bromophenol blue in 5 ml acetone.

Solution 2: 0.5gms of Silver nitrate in 50 ml of distilled water.

Took out 15 ml from solution 2 and make its volume up to 50 ml using acetone. Now add 45 ml of solution 2 to solution 1, which is called bromo-phenol blue solution.

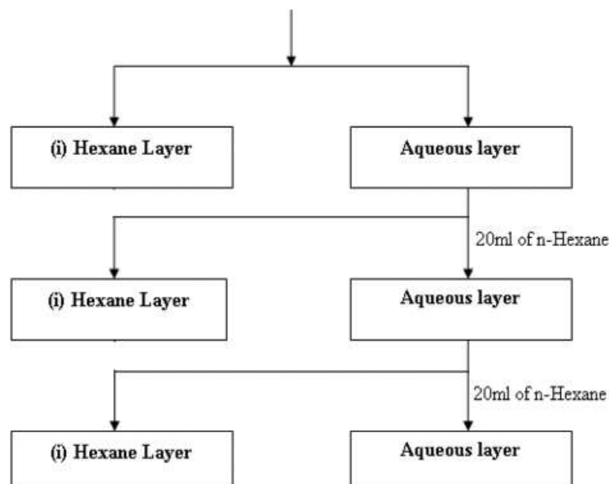
Extraction of Triazophos Pesticide from Blood[9,12-15]

4 ml blood + 6 ml of 10% Sodium tungstate solution + 15 ml of sulphuric acid, shaken for two minutes



Filterate (i), (ii) & (iii) are pooled + 15ml of n-Hexane is taken in a separating funnel, shaken for two minutes and separated by two layers.

Hexane layers (i), (ii) & (iii) are pooled and passed through the Sodium sulphate and evaporated up to 1ml.



Preparation of TLC Plates

TLC plates were prepared by dissolving 25 gm of silica gel G in 50 ml of distilled water to make slurry. This slurry was poured on the applicator and the applicator was then moved over the plate in one motion. Plates were allowed to dry at room temperature and then kept in hot air oven at 80°C for one hour.

Spotting and Development of Plates

Pesticide extracted from blood was loaded on TLC plates along with the standard with appropriate markings. Spotted plates were developed in different solvent system taken in different ratios. After developing, the TLC plates were taken out from the solvent chamber and air-dried.

Visualization of TLC Plate

The dried and developed TLC plates were sprayed with Bromophenol blue followed by 4% Acetic acid.

Results and Discussion

After the development the TLC plates are sprayed with spraying reagent such as Bromophenol blue followed by 4% Acetic acid. There were 9 different solvent systems with different volumetric ratios. The R_f value of Triazophos extracted from blood under experimental conditions was found nearly equal to standard used in some cases. The retentions factor (Rf) is defined as the ratio of the distance moved by analyte from the origin to the distance moved by solvent from the origin.The response of separation of Triazophos in all 9 solvents was analysed and presented in Tables 1,2 ,3,4,5 and 6.

Table 1: TLC parameters in solvent system Acetone : Benzene

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
1:9	12	6.6	6.5	7	0.94	0.928
2:8	16	6.2	6	7.5	0.826	0.800
3:7	13	6.8	6.2	7.5	0.906	0.826
4:6	11	8.1	7.4	8.3	0.975	0.891
5:5	15	6.7	6.4	7.2	0.93	0.888
6:4	12	7.7	7	7.8	0.987	0.897
7:3	11	7.4	7.3	7.7	0.961	0.948
8:2	12	7.8	7.5	8.3	0.939	0.903
9:1	15	8.1	8	8.2	0.987	0.975
pure benzene	20	no result	-	7.7	-	-

Table 2: TLC parameters in solvent system Ethyl acetate: Hexane

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
1:9	13	6.7	7.3	8.6	0.77	0.848
2:8	12	6.8	7.5	8.5	0.8	0.882
3:7	17	5	7.2	7.5	0.68	0.96
4:6	14	5.9	7.1	8	0.737	0.887
5:5	14	6.8	7	7.5	0.906	0.93
6:4	17	7.3	7.3	7.7	0.948	0.94
7:3	12	no result	-	7.8	no result	-
8:2	14	no result	-	8.5	no result	-
9:1	15	no result	-	8.4	no result	-

Table 3: TLC parameters in solvent system Dichloromethane: Chloroform

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
9:1	17	3.8	7	7.9	0.481	0.888
8:2	18	3.2	6.5	8.7	0.367	0.747
7:3	23	3.9	7.2	8.5	0.458	0.847
6:4	16	2.8	7.3	8.1	0.345	0.901
5:5	18	2.5	7.3	8.3	0.301	0.879
4:6	18	3.3	7.5	8.7	0.379	0.862
3: 7	17	4.1	6.9	8.5	0.482	0.811
2:8	21	2.8	7	8.5	0.329	0.823
1:9	20	3.7	7.7	8.8	0.42	0.871

Table 4: TLC parameters in solvent system dichloromethane

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
100	17	3.2	7.4	7.9	0.405	0.936

Table 5: TLC parameters in solvent system Benzene: chloroform

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
8:2	17	7.6	7.2	8.5	0.894	0.847

Table 6: TLC parameters in solvent system benzene: methanol

Ratio	Time (Mins)	Distance Travelled (Centimetre) Standard	Sample	Solvent	R_f (Standard)	R_f (Sample)
8:2	18	7.7	7.3	8.2	0.939	0.890

Table 7: Solvent Systems of best Volumetric Ratios with R_f values

S. No.	Solvent system	Ratio	R_f (Standard)	R_f (Sample)
1	Acetone :Benzene	9:1	0.980	0.970
2	Ethylacetate :Hexane	6:4	0.945	0.942
3	Dichloromethane :Chloroform	3:7	0.480	0.481
4	Dichloromethane	10	0.400	0.401
5	Benzene: chloroform	8:2	0.895	0.846
6	Benzene: Methanol	8:2	0.939	0.890
7	Petroleum benzene (pure)	no result	_	_
8	Dichloromethane: Water	no result	_	_
9	Dichloromethane: Propanol	no result	_	_

Conclusion

The long time involved in screening of the Triazophos with analytical methods like GC, HPLC etc. an alternative method, TLC was searched[12-15]. This is a simple method, easy to perform, no machine is required, takes less time to analyse the sample. The solvent systems of best volume ratio with R_f values of standard samples are given in Table 7.

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Effects of Temperature & Putrefaction on the Analysis of Carbofuran & Carbaryl Insecticides

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Abstract

Pesticides are frequently encountered in forensic toxicology division of FSLs. Due to pendency of medico-legal cases; biological samples are often stored for some time from the time of collection before analyses. The role of temperature and putrefaction that may inevitably interfere with analyses results is often ignored in such cases. In our preliminary study, we have tried to produce evidence for difference in thin layer chromatography results of two insecticides- carbofuran and carbaryl as a direct consequence of changes in preservation methods of samples. This subject must further be explored taking into consideration factors like temperature, humidity, time duration, etc. so as to support research on better preservation methodology of forensic samples.

Keywords: Carbofuran; Carbaryl; Chromatography; Putrefaction; Forensic Science Laboratories (FSLs).

Introduction

The use of pesticides for criminal purposes has not only increased but their analyses complexities have also grown over decades. Insecticides have largely been abused for suicides/ homicides because of their ease of availability in regular household work like controlling mosquitoes, cockroaches, bugs, flies, etc. Deaths in suspicious circumstances are reported as medico-legal death cases and the post mortem samples are forwarded to Forensic Science Laboratories (FSLs) for analyses. Forensic Toxicology is a special area of analytical chemistry that deals with analyses of poisons in samples like blood, viscera, body fluids, etc. There are a number of factors responsible for affecting the analytical results and therefore, no standardized protocols for the identification of poisons in biological samples can be followed. In this study, we have made an effort to study the putrefaction of biological samples on the analysis of two insecticides- Carbofuran and Carbaryl.

Materials and Methods

Materials Used

HPTLC plates, Chloroform, Acetic acid, Acetonitrile were purchased from Merck Ltd. Mumbai. Methanol, n-Hexane, Acetone, Benzene were purchased from Glaxo India Ltd. Mumbai. All chemicals were of HPLC grade.

Insecticide Standards

Carbofuran and Carbaryl standards (Technical

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grade) were prepared in acetone at concentrations 0.01, 0.05, 0.10, 0.50 & 1.00 mg/ml for calibration. For spiking in biological samples, standards were prepared separately in acetone at concentration 1mg/ml.

Sample Preparation

Biological Tissue samples (goat liver)- 50g each were taken in separate beakers and labeled as RT1, RT2, LT1 and LT2. Samples RT1 <1 were spiked with 2ml standard solution of Carbofuran at a concentration of 1mg/ml. Similarly, RT2 & LT2 were spiked with 2ml standard solution of Carbaryl at a concentration of 1mg/ml. RT1 & RT2 were kept at 37°C for 10 days without covering with lid and analyzed as putrefied samples. LT1 & LT2 were covered with aluminum foil and kept in the refrigerator in dark at 4°C for 10 days and analyzed as preserved samples.

Extraction & Purification

The samples were homogenized and refluxed with n-hexane (50ml) on a hot water bath for 90 minutes. The contents were cooled and filtered and the residue extracted twice with n-hexane (25ml) saturated with acetonitrile. The extract was dehydrated and purified by passing through sodium sulphate and silica gel-G column and evaporated to dryness on a water bath. The residue was reconstituted in 1ml n-hexane and TLC was performed with the extracted and purified sample.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC plates were activated at 110°C for 30 minutes and then cooled to room temperature before analysis. Spotting of standard solutions and extracted samples was done by HPTLC sample applicator (Desaga-AS-30, Germany). The plates were placed in developing chamber and different solvent systems of hexane and acetone were tested. After elution of spots, the plates were air dried and scanned by mutiwavelength program and λ_{max} values were noted for specific densitograms of each insectide on Desaga-Densitometer-CD20, Germany.

Results and Discussion

The extraction of insecticides from biological matrix like viscera is difficult due to interferences from fat, degraded protein, coloring matter, etc. Out of the several solvents tried, it was observed that the % recoveries of insecticides from visceral tissue were maximum for hexane as extracting solvent (85-90%) at microgram level. The recoveries may further be increased by using solid phase extraction (SPE) technique to detect the insecticides at nanogram level.

Table 1: A comparison of putrefied & preserved samples

Solvent System Used	Insecticide	Sample Name	Condition of Sample	Rf values
Hexane : Acetone (9:1)	Carbofuran	Std	Standard Solution	--
		RT1	Putrefied	--
		LT1	Preserved	--
	Carbaryl	Std	Standard Solution	Spot A= 0.42
		RT2	Putrefied	Spot A= 0.41 Spot B= 0.45
		LT2	Preserved	Spot A= 0.41 Spot B= 0.43
Hexane : Acetone (8:2)	**Carbofuran**	**Std**	**Standard Solution**	**0.53**
		RT1	Putrefied	0.61
		LT1	Preserved	0.56
	Carbaryl	Std	Standard Solution	Spot A= 0.41
		RT2	Putrefied	Spot A= 0.41 Spot B= 0.43
		LT2	Preserved	Spot A= 0.41 Spot B= 0.49

Hexane : Acetone (7:3)	Carbofuran	Std	Standard Solution	0.50
		RT1	Putrefied	0.62
		LT1	Preserved	0.55
	Carbaryl	Std	Standard Solution	Spot A= 0.41
		RT2	Putrefied	Spot A= 0.45 Spot B= 0.43
		LT2	Preserved	Spot A= 0.41 Spot B= 0.42

Various solvent systems that were used for HPTLC showed that the Rf values varied with polarity of the solvents used and thus the choice of solvent for a particular pesticide is very important. It was observed that there was a difference in Rf values of putrefied (kept at 37°C) and preserved (kept at 4°C) samples in all the three solvent systems tested (Table 1). In case of Carbofuran, the putrefied sample RT1 showed higher Rf value as compared to preserved sample LT1. In case of Carbaryl, the number of spots observed for putrefied sample RT2 was more than preserved sample LT2. The possible reason may be degradation of the insecticide due to high temperature and/or exposure to daylight. These results were also supported by HPTLC densitograms where more than one peak was observed for putrefied sample RT2. The results of HPTLC also show that below 0.01mg/ml, neither of the insecticides could be detected at their respective λ_{max}.

Conclusion

The effects of temperature and/or daylight on sample condition and analyses are very significant as the analyte of interest present in the biological matrix may be degraded by the activity of various metabolizing enzymes over a period of time. This subject needs further study for longer time durations so as to better understand the performance of putrefied and preserved samples as their analytical profiles show differences. The study needs to be replicated on other classes of pesticides as well that are commonly encountered in forensic investigations.

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Original Article

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Detection and Identification of Chlorophenraminemaleate in Street Narcotic Stuff

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Abstract

Detection and identification of diluents in common drugs samples is frequent and challenging task for forensic chemists. Appearance of new drugs or combination of new drugs and misuse/ abuse of drugs, used for medical purposes, not only has substantial and detrimental effect on the health and welfare of the people and also generates several serious sociological problems. The present work describes the detection and identification of uncommon diluent's Chlorophenraminemaleate in narcotic materials by chemical colour tests, thin layer chromatographic and U. V. spectroscopy.

Keywords: Chlorophenraminemaleate; CPM; TLC; U.V.

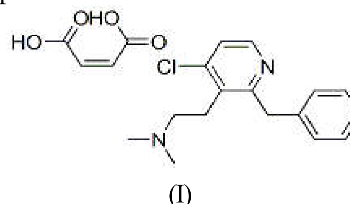
Introduction

Pure heroin is quite expensive and hence clandestine laboratories di acetylates either opium as such or crude morphine. The resultant product is brown in colour. This crude heroin is colloquially known as brown sugar, smack, gard etc. brown sugar is most widely abused drug. It contains heroin and other opium alkaloids like papaverine, narcotine, and acetyl codeine, acetyl morphine etc. and active adulterant like caffeine, methaqualone, phenobarbitone, diazepam, paracetamol etc. for prosecution under NDPS act 1985 only the identification of heroin in brown sugar is required.

The increase in frequency of narcotic drugs and number of addicts pose a maiming situation for law enforcement authority as well as the scientific staff involved. The ingenuity of the illicit street samples of drug produced by the clandestine laboratories are normally concoction mixture which require modern analytical techniques that are rapid and sensitive for the unambiguous identification of these drugs [1-4].

The present work describes the detection and identification of Chlorophenraminemaleate, in the exhibit confiscated from accused by chemical colour test, thin layer chromatography and U.V. spectroscopy.

Chlorophenraminemaleate [5] is (RS)-3-(4-chlorophenyl)-3-(2-pyridyl) pro pyl dimethylamine hydrogen maleate used as antihistaminic compound having molecular weight 390.87, m.p. 132-135°C white crystalline powder with molecular formula $C_{16}H_{19}N_2ClC_4H_4O_4$ freely soluble in water, ethanol and chloroform the chemical structure of Chlorophenraminemaleate is



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Brief Study of Case

Bhopal police arrested a person, which were engaged in taking illicit narcotic substance and seized several exhibits like cigarette foil, match box, paper packets along with brown powder a vial syringe with needle.

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On medical examination doctor found several prick marks and blackish blue injuries along the cubical vein and its tributaries and the area thromboses on both hand upper cubical areas along with old wounds on legs and doctor opine that these self inflicted prick marks of injection on both cubical areas are consistent with history of drug abuse. Hence blood and urine preserved for forensic examination for chemical analysis in forensic science laboratory.

Materials and Methods [6-9]

All chemicals used were of AR grade dried and purified before use, double distilled water used as required for TLC the sample was dissolved in chloroform (about 1mg/ml). Control drug sample Chlorophenraminemaleate were purchased from local medical shop.

Thin Layer Chromatographic Analysis

A standard glass TLC plates was coated with slurry of silica gel G in water to a uniform thickness of 0.25 mm. heating in an oven at 110°C for about one hour activated the plate. An aliquots of standard Chlorophenraminemaleate and extract obtained were spotted on to the plate, which was developed with Cyclohexane: toluene: diethyl amine (75:15:10) in a pre saturated TLC chamber, to a height of 10 cm. The plate was removed from the chamber dried in air and sprayed by dragendroffs reagent at which gave orange coloured spots. The RF value of Chlorophenraminemaleate 0.35 can be compared with the obtained spots of extract. The RF value of Chlorophenraminemaleate in different solvent systems are given in following table

Table 1:

S. No.	Solvent system	Rf value
1.	Methanol/ Strong Ammonia (100:1.5)	0.45
2.	Acetone	0.30
3.	Chloroform/Methanol (90:10)	0.13
4.	Methanol	0.14
5.	Cyclohexane/Toluene/Diethylamine (75:15:10)	0.35

Colour Test

To a solution of Chlorophenraminemaleate add 3 ml of water and 1 ml of 10 M NaOH and extract with 5 ml ether 3 times. To 0.1 ml of the aqueous layer 10 mg resorcinol solution and 3ml of sulphuric acid is added and heated in water bath for 15 min the solution remains colourless. To the remainder of the aqueous layer when 2 ml of bromine solution is added and solution is heated on water bath for 15 minutes up to boiling and cooled when in this solution 10mg of resorcinol solution and 3 ml of sulphuric acid is added and heated in boiling water bath for 15 minutes it develops blue colour.

UV Spectroscopy

The UV spectra were taken as Shimadzu UV spectrophotometer model 2550. The extractives showed ë max in aqueous acid at 261nm and 266 nm tallied with the control sample.

Results and Discussion

The TLC experiments indicating the presence of diacetyl morphine along with another organic nitrogenous compound. The isolation and identification of drug by TLC technique, colour test and concordantly confirmed by U.V. spectral studies showed that drug were isolated. TLC coupled with U.V. spectroscopy between 200nm to 400nm provided a reliable quick method of detection and identification of Chlorophenraminemaleate drug used as anti allergic agent which is evident from recorded observation a sharp ë max in aqueous acid at 261nm and 266 nm in extracts tallied with standard sample, concluded that chlorphenaminmaleat drug. Such rapid resolution by TLC, colour tests and U.V. spectroscopy will undoubtly is of great value to the analytical chemist who may be confronted with the analysis of this drug in different materials referred in analysis of narcotic substances.

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Screening/Spot/Colour Test of Anti-Depressants

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Abstract

Antidepressants are class of drugs that reduce symptoms of depressive disorders by correctly chemical imbalances of neurotransmitters in the brain. These imbalances may be responsible for changes in mood and behaviour of an individual. In forensic autopsy case, the forensic pathologist may require a complete toxicological investigation for different poisons including Anti-depressents. In India, Forensic Science Laboratories run by Government under the Home ministry usually carry out this The samples have to be analyzed by the forensic Toxicologist/Chemists/ Scientist. This article deals with the screening/spot test for antidepressants in a step-wise manner, which can be of handy reference for the forensic toxicologist.This article is in continuation of toxicology manual series-XVIII, screening/spot test of aphrodisiacs (sex drugs), International Journal of Medical Toxicology & Legal Medicine,Vol.14, No.3 Jan-March 2012.

Keywords: Antidepressants; Screening; Colour; Spot Test etc.

Introduction

Antidepressants are the drugs that are used to relieve the symptoms of depression. They were first developed in the 1950's and have been used on regular basis. Initially used for the treatment of depression in addition to improving one's mood as well as behaviour. The intake of antidepressants enhances the activity of certain chemicals that work in our brain known as neuro transmitters. They pass on the signals from one cell to another. These drugs are advised to the patients suffering from moderate to severe depression illness, severe anxiety, panic attacks, obsessive compulsive disorders and post traumatic stress disorder. They are basically classified on the basis of which chemicals in the brain they affect [1-2].

We have tried to set out standard procedures for screening/spot test for alkaloids which are easily available and useful for the forensic science laboratory. This article covers the spot test/colour test of anti-depressants such as amitriptyline, butriptyline, clomipramine, desipramine, dibenzepin, dosulepin, doxepin, imipramine, iproniazid, isocarboxazid, mebarazine, mianserin, nialamide, nomifensive, nortriptyline, noxiptiline, opipramol, phenelzine, protriptylin, tofenacin, trazodone, trimipramine, viloxazine and zimeldin etc [3-9].

Amitriptyline

Marquis test

1. Two ml of extract is taken in test tube.
2. Few drops of marquis reagent are added to it.
3. Brown to orange colour is observed which indicates the presence of amitriptyline.

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Table 1: Classification of antidepressants

S. no	Types	General Information	Examples
1.	Monoamine oxidase inhibition	It is given those individuals who don't respond to any other antidepressants. It must not be taken with certain foods, beverages and medications can cause dangerous interactions.	Phenelzine, tranylcypromine
2.	Tricyclic antidepressants drugs	TCAs are rapidly absorbed from the Digestive tract, bind to plasma proteins, and become widely distributed in tissues as a result of their lipophilic nature. It affects the neurotransmitters norepinephine & serotonin.	Amitryptyline, Imipramine, Clomipramine
3.	Selective serotonin reuptake inhibitors	It affects the serotonin levels in the brain. Serotonin is a chemical neurotransmitters used to treat depression.	Fluoxetine, Sertraline, Citalopram
4.	Serotonin & norepinephrin reuptake inhibitors	It works on two chemical neurotransmitters serotonin as well as norepinephrin. It is used to treat the major depression as well as mood disorders.	Duloxetine, velafaxine
5.	Norepinephrin & dopamine reuptake inhibitors	It works on increasing the level of norepinephrin and dopamine to prevent their reabsorption into the cell.	Buproprion
6.	Combined reuptake inhibitors and receptor blockers	It is useful in two ways firstly it prevents the reabsorption into the nerve cell and at the same time blocking the nerve cell receptors	Trazidone, maprotiline

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Colour changes from brown to green which indicates the presence of amitriptyline.

Sulphuric Acid Test

- Few drops of extract are taken on a white tile.
- Few drops of sulphuric acid are added to it.
- Orange colour is observed which indicates the presence of amitriptyline.

Butriptyline

Marquis test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Violet colour is observed which indicates the presence of butriptyline.

Clomipramine

Forrest Test

- One to two ml of extract is taken in test tube.
- Few drops of forrest reagent are added to it.
- Blue colour is observed which indicates the presence of clomipramine.

FPN test

- Two ml of extract is taken in test tube.
- Few drops of FPN reagent are added to it.
- Blue colour is observed which indicates the presence of clomipramine.

Mandelin's test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Blue colour is observed which indicates the presence of clomipramine.

Libermann's test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Blue colour is observed which indicates the presence of clomipramine.

Desipramine

Forrest Test

- One to two ml of extract is taken in test tube.
- Few drops of forrest reagent are added to it.

- Blue colour is observed which indicates the presence of desipramine.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Colour changes from yellow to blue which indicates the presence of desipramine.

Dibenzepin

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Green colour is observed which indicates the presence of dibenzepin.

Dosulepin

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Reddish brown colour is observed which indicates the presence of dosulepin.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Green colour is observed which indicates the presence of dosulepin.

Marquis Test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Brown colour is observed which indicates the presence of dosulepin.

Sulphuric Acid Test

- Few drops of extract are taken on a spotting tile.
- Few drops of sulphuric acid are added to it.
- Violet colour is observed which indicates the presence of dosulepin.

Doxepin

Libermann's test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Black colour is observed which indicates the presence of doxepin.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Brown colour is observed which indicates the presence of doxepin.

Marquis Test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Brown colour is observed which indicates the presence of doxepin.

Sulphuric Acid Test

- Few drops of extract are taken on a white tile.
- Few drops of sulphuric acid are added to it.
- Orange colour is observed which indicates the presence of doxepin.

Imipramine

Forrest Test

- One to two ml of extract is taken in test tube.
- Few drops of forrest reagent are added to it.
- Blue colour is observed which indicates the presence of imipramine.

FPN Test

- One to two ml of extract is taken in test tube.
- Few drops of FPN reagent are added to it.
- Blue colour is observed which indicates the presence of imipramine.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Blue colour is observed which indicates the presence of imipramine.

Libermann's Test

- One to two ml of extract is taken in test tube.

- Few drops of libermann's reagent are added to it.
- Blue colour is observed which indicates the presence of imipramine.

Iproniazid

Cyanogen Bromide Test

- One to two ml of extract is taken in test tube.
- Few drops of cyanogens bromide reagent are added to it.
- Orange to pink colour is observed which indicates the presence of iproniazid.

Nessler's Test

- Two to three drops of extract is taken in a porcelain basin.
- Two to three drops of nessler's reagent is added to it.
- Agitated and heated the mixture at 100! in water bath.
- Black colour is observed which indicates the presence of iproniazid.

Isocarboxazid

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Red to orange colour is observed which indicates the presence of isocarboxazid.

Nessler's test

- Two to three drops of extract is taken in a porcelain basin.
- Two to three drops of nessler's reagent is added to it.
- Agitated and heated the mixture at 100! in water bath.
- Black colour is observed which indicates the presence of isocarboxazid.

Mebarazine

Palladium chloride test

- Two ml of extract is taken in test tube.
- One ml of palladium chloride solution is added to it.
- Above solution is heated for two minutes.
- Black colour is observed which indicates the presence of mebarazine.

Sulphuric Acid Test

- Few drops of extract are taken on a white tile.
- Few drops of sulphuric acid are added to it.
- Orange colour is observed which indicates the presence of mebarazine.

Mianserin

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Violet colour is observed which indicates the presence of mianserin.

Libermann's Test

- One ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Violet colour is observed which indicates the presence of mianserin.

Nialamide

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Red colour is observed which indicates the presence of nialamide.

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Reddish orange colour is observed which indicates the presence of nialamide.

Nessler's Test

- Two to three drops of extract is taken in a porcelain basin.
- Two to three drops of nessler's reagent is added to it.

- Agitated and heated the mixture at 100! in water bath.
- Black colour is observed which indicates the presence of nialamide.

Nomifensine

Coniferyl Alcohol Test

- Few drop of extract is taken in a test tube.
- A drop of coniferyl alcohol is added on it.
- Yellow colour is observed which indicates the presence of nomifensine.

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Orange colour is observed which indicates the presence of nomifensine.

Marquis Test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Brown colour is observed which indicates the presence of nomifensine.

Nortriptylin

Marquis Test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Orange colour is observed which indicates the presence of nortriptyline.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Colour changes from brown to green which indicates the presence of nortriptyline.

Sulphuric Acid Test

- Few drops of extract are taken on a white tile.
- Few drops of sulphuric acid are added to it.
- Orange colour is observed which indicates the presence of nortriptyline.

Noxiptiline

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Black colour is observed which indicates the presence of noxiptiline.

Opipramol

Forrest Test

- One to two ml of extract is taken in test tube.
- Few drops of forrest reagent are added to it.
- Blue colour is observed which indicates the presence of opipramol.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Colour changes from brown to green which indicates the presence of opipramol.

Phenelzine

Benedict's Test

- One ml of extract is taken in a test tube.
- 0.5 ml of Benedict's reagent is added to it.
- Solution is heated at 100! for 3 mins.
- Orange colour is observed which indicates the presence of phenelzine.

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Orange colour is observed which indicates the presence of phenelzine.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Brown colour is observed which indicates the presence of phenelzine.

Nessler's Test

- Two to three drops of extract is taken in a

porcelain basin.

- Two to three drops of nessler's reagent is added to it.
- Agitated and heated the mixture at 100! in water bath.
- Black colour is observed which indicates the presence of phenelzine.

Palladium Chloride Test

- Two ml of extract is taken in test tube.
- One ml of palladium chloride solution is added to it.
- Solution is heated for 2 mins.
- Black colour is observed which indicates the presence of phenelzine

Protriptylin

Marquis Test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Green colour is observed which indicates the presence of protriptyline.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Colour changes from violet to brown which indicates the presence of protriptyline.

Sulphuric Acid Test

- Few drops of extract are taken on a white tile.
- Few drops of sulphuric acid are added to it.
- Green colour is observed which indicates the presence of protriptyline.

Tofenacine

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Reddish orange colour is observed which indicates the presence of tofenacine.

Marquis Test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Yellow colour is observed which indicates the presence of tofenacine.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Yellow colour is observed which indicates the presence of tofenacine.

Sulphuric Acid Test

- Few drops of extract are taken on a white tile.
- Few drops of sulphuric acid are added to it.
- Orange colour is observed which indicates the presence of tofenacine.

Trazodone

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Violet colour is observed which indicates the presence of trazodone.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Grey to violet colour is observed which indicates the presence of trazodone.

Trimipramine

Forrest Test

- One to two ml of extract is taken in test tube.
- Few drops of forrest reagent are added to it.
- Blue colour is observed which indicates the presence of trimipramine.

FPN Test

- One to two ml of extract is taken in test tube.
- Few drops of FPN reagent are added to it.
- Blue colour is observed which indicates the presence of trimipramine.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Blue colour is observed which indicates the presence of trimipramine.

Viloxazine

Libermann's Test

- One to two ml of extract is taken in test tube.
- Few drops of libermann's reagent are added to it.
- Black colour is observed which indicates the presence of viloxazine.

Mandelin's Test

- Two ml of extract is taken in test tube.
- Few drops of mandelin's reagent are added to it.
- Blue to green colour is observed which indicates the presence of viloxazine.

Marquis test

- Two ml of extract is taken in test tube.
- Few drops of marquis reagent are added to it.
- Yellow colour is observed which indicates the presence of viloxazine.

Zimeldin

Cyanogen Bromide Test

- One to two ml of extract is taken in test tube.
- Few drops of cyanogens bromide reagent are added to it.
- Red colour is observed which indicates the presence of zimeldin

Libermann's Test

1. One to two ml of extract is taken in test tube.
2. Few drops of libermann's reagent are added to it.
3. Brown colour is observed which indicates the presence of zimledin.

Preparation of Solutions/ Reagents

Benedict's Reagent

a. 1.73 g of copper sulphate is dissolved in 10 ml of water.

b. 17.3 g of trisodium citrate and 10 g of anhydrous sodium carbonate are dissolved in 80 ml water with heating Solution b is poured in solution a and diluted the mixture up to 100 ml with water.

Coniferyl Alcohol Reagent

0.1 g of coniferyl alcohol is warmed until it melts, dissolved in 3 ml of ethanol and diluted to 10 ml with ethanol.

Forrest Reagent

Equal volumes of a 0.2 % (w/v) solution of potassium dichromate, 30 % (w/v) solution of sulphuric acid, 20 % (w/v) solution of perchloric acid and 50 % solution of nitric acid are mixed.

FPN Reagent

5 ml of 5 % (w/v) ferric chloride solution, 45 ml of 20 % (w/w) solution of perchloric acid and 50 ml of 50 % (v/v) solution of nitric acid are mixed.

Libermann's Reagent

1 gm of sodium or potassium nitrite is dissolve in 10 ml of sulphuric acid with cooling and swirling to absorb the brown fumes.

Mandelin's Reagent

1 g of ammonium vanadate is dissolved in 1.5 ml of water and diluted upto 100 ml with concentrated sulphuric acid.

Marquis Reagent

100 ml of concentrated sulphuric acid is mixed with 1 ml of 40% (v/v) formaldehyde solution.

Nessler's Reagent

a. 50 g of mercuric chloride and 35 g of potassium iodide is dissolved in 200 ml of water and cooled.

b. 50 g of sodium hydroxide is dissolved in 250 ml of water and cooled.

c. The cold solutions of a and b are mixed and made up to 500 ml. The mixture is allowed to stand and decant the clear supernatant liquid for use.

Conclusion

In any analysis of poison, screening/spot test is very useful for knowing the presence of the anti-depressants which can be confirmed by the more confirmatory tests.It saves time for the toxicologist in ruling out the poisons and gives a quick clue to the doctors for patient management in emergency poisoning cases. The result of the analytical methods depends on the amount and purity of the sample extracted. It is important for the forensic toxicologists to know the best available method and help to detect the poison in the crime investigations.

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Phenol Poisoning with Analytical Aspects and Its Management

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Abstract

Phenol or carbolic acid has been used in households since time immemorial. It has also been used extensively for medicinal purposes since Lister first published his paper titled 'On the Antiseptic Principle in the Practice of Surgery'. With time, the side effects of using phenol have also been noticed, resulting in decreased medicinal use of phenol. However, continued household use of phenol and its derivatives makes it one of the most common cause of medico-legal presentation in casualty.

This paper has been written with the aim of highlighting the presentation and management with focus on analytical aspects of phenol poisoning.

Keywords: Phenol; Poisoning; Analysis; Management.

Introduction

Phenol or carbolic acid [Hydroxybenzene, Phenylic acid, Phenylic alcohol, C_6H_5OH] [1-3] was first extracted from coal tar in 1834 [4]. The use of phenol in surgery by Sir Joseph Lister resulted in drastic fall in fatalities [5]. In 1867 Lister published his landmark paper 'On the Antiseptic Principle in the Practice of Surgery' [6] and it proved to be a turning point in health care [7]. Subsequently the use of carbolic acid was done in treating conditions like typhoid fever, mucous tubercles, primary syphilitic sores, psoriasis, pityriasis, prurigo and chronic bronchitis [8,9]. However, there were numerous instances, when the use of carbolic acid during treatment resulted in toxicity, leading to a limited use of phenol. Phenol is one of the most common household poisons. In one study done by Patil A et al it was concluded that Phenol poisoning was the most common cause of medico-legal presentation in casualty of Navi Mumbai hospital, India [10].

Sources of Carbolic Acid

1. Antiseptic and disinfectant especially for sterilising floors, walls, furnishing, glassware and instruments [3].
2. Phenol is used in the production of resins, which are used in the plywood, construction, automotive, and appliance industries [11].
3. It is used for manufacture of plastics [11].
4. It is also used in preservatives.
5. Medical Sources [3] are
    a. Face peel in plastic surgery.
    b. Neurolysis for spasticity.
    c. Treatment of localised skin disorders (Castellani's paint) and as a local anaesthetic.
    d. Ear and nose drops, throat lozenges, and mouthwashes.
6. Cresol which is derivatives of phenol is used as

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disinfectant and antiseptic [12,13].

7. Creosote – is a mixture of phenols and consists mainly of cresol and guiacol. It is used as household remedy for coughs and is found in many proprietary preparations [12,13].
8. Resorcinol which is a derivative of phenol is a colourless crystalline substance and is used for the treatment of various skin diseases including ringworm, psoriasis, eczema etc [12,13].
9. Dettol is chlorinated phenol; parachlorometaxylenol. Although it is said to be practically nontoxic in adults, deaths have still been reported [13-15].
10. Thymol is an alkyl derivative of phenol obtained from volatile oils of thymus vulgaris, Monarda punctata or Trachyspermum ammi. It occurs in colourless crystals with characteristic pungent odour and taste. It was used earlier as an antihelminthic, antifungal and antiseptic [13,14].

Exposure of Phenol

Phenol is a pure acid which consists of short, colourless, prismatic, needle like crystals [4,13]. These crystals turn pink on exposure to air [2,4]. Phenol has a characteristic phenolic or carbolic odour or hospital odour [3,13]. It is sparingly soluble in water, but freely soluble in alcohol, benzene, ether, glycerine and fat and hence can attack the nervous system [13]. It is not a true acid as it does not turn litmus paper red [13]. Common routes of exposure are usually inhalational, oral and dermal. Few Points about Exposure of Phenol are

1. Breathing air contaminated with phenol.
2. Drinking water contaminated with phenol. Phenol in soil usually drains into groundwater.
3. Overdose of phenol and its derivatives during treatment.
4. Accidental ingestion of phenol instead of another liquid like cough remedy/ medicine.
5. Breathing air at work sites like petroleum industry, manufacture of nylon, epoxy resins and polycarbonates, herbicides, wood preservatives, hydraulic fluids, heavy-duty surfactants, lube-oil additives, tank linings and coatings, and intermediates for plasticizers and other specialty chemicals [11].
6. Breathing fumes while treating a patient.
7. Dermal contact by products containing phenol.
8. Eating food materials like smoked summer sausage, smoked pork belly, mountain cheese, fried bacon, fried chicken, and black fermented tea, where a low level of phenol is found [11].

Pharmacokinetics

Mechanism of Action

Phenol is a protoplasmic poison. Because it is an irritant, tissue damage, inflammation, or other irritation effects may occur at the sites of absorption. Phenol impairs the stratum corneum and produces coagulation necrosis by denaturing and precipitating proteins. It causes widespread capillary damage and clotting in superficial blood vessels. It also causes central nervous system depression, metabolic acidosis and renal damage. The toxicity is due to phenol and the metabolites formed during the metabolism of phenol i.e. hydroquinone, catechol, and benzoquinone. These three metabolites exhibit potency similar to phenol [2-4,11,13,16].

Metabolism

Phenol is readily absorbed from all routes i.e. rectum, gastrointestinal tract, respiratory tract, serous cavities, skin, vagina and wounds [1,2,12-14,16]. Conjugation with glucuronic acid and conjugation with sulfate are the main routes of detoxification of phenol. Phenol is excreted in the urine as phenyl glucuronide and phenyl sulfate metabolites [2]. Phenol is metabolised to pyrocatechol and hydroquinone in the liver. These metabolites are also excreted in the urine. These get oxidized in air and because of this, initially colourless urine turns to greenish or blackish on standing (Carboluria). Phenol is also excreted by lungs, salivary glands, stomach and skin. There is no information on levels of phenol in human breast milk [11]. The time required for complete excretion is 36 hours.

Phenol in Environment

Phenol enters the air, water, and soil as a result of its manufacture and use. Phenol has a short half-life in air, less than 1 day. In air, it reacts with photochemically produced hydroxyl radicals. Phenol generally remains in soil only about 2–5 days. In soil, phenol biodegrades under both aerobic and anaerobic conditions. Phenol is rapidly degraded in water, but it can remain in water for upto 9 days. Phenol does not accumulate in fish, other animals, or in plants [17,18].

Exposure Limit for Phenol

NIOSH REL - TWA 5ppm (19mg/m3) C 15.6 ppm

(60 mg/m3), OSHA PEL - TWA 5ppm (19mg/m3). The biological exposure index (BEI) for occupational exposure to 5 ppm phenol is 250 mg total phenol in urine/g creatinine. Environment Protection Agency has determined that exposure to phenol in drinking water at a concentration of 6 milligrams per liter (mg/L) for up to 10 days is not expected to cause any adverse effects in a child. EPA has determined that lifetime exposure to 2 mg/L phenol in drinking water is not expected to cause any adverse effects. The FDA has determined that the phenol concentration in bottled drinking water should not exceed 0.001 mg/L. [1, 11, 17, 18]

Fatal Dose and Fatal Period

***Fatal Dose*:** Literature report of human LD_{LO} by the oral route range from 0.14 to 14 g/Kg. [1,18]. U.S. Department of health and human services records that the minimal lethal oral dose of phenol is approximately 70 mg/kg in adults. Other estimates indicate that an oral dose as low as 1,000 mg could be fatal in humans, but patients occasionally survived doses as high as 65,000 mg [11].

***Fatal Period*:** Fatal period of phenol is 3-4 hours. [4, 12-14, 16]

Normal / Reference Values

Phenol is a normal constituent of urine. The total phenol concentration in the urine of unexposed individuals does not exceed 20 mg/L and the mean is usually <10 mg/L.[11, 17]

Signs and Symptoms of Phenol Poisoning (Carbolism) [2-4, 12-14, 16, 19-24]

Signs and symptoms of phenol poisoning are detailed in Table 1.

Table 1: Signs and symptoms of phenol poisoning

		Features
Acute poisoning	Local effects	• Skin: Numbness, burns (heal leaving a brown stain)
		• GI tract: nausea, vomiting, pain, lips & mouth & tongue are corroded, deglutition and speech becomes difficult.
	Systemic effects	• General – Odour in breadth *(phenolic)*, pupils - miotic, breathing - stertorous, pulse -feeble, irregular and rapid, face covered with cold sweat, dusky cyanosis.
		• CNS - Initial stimulation followed by depression especially of respiratory centres.
		• CVS - Hypotension, tachycardia, arrhythmias, infrequent gasps, laryngeal and pulmonary edema, bronchitis, bronchopneumonia.
		• Hepatorenal – oliguria, carboluria, renal and hepatic failure
		• Blood- hemolysis, methemoglobinemia
		• Metabolic acidosis and respiratory alkalosis
		• **Rabbit syndrome**- fine rapid rhythmic contractions of the perioral musculature resembling the chewing movement of rabbit.
		• Lock jaw
Chronic poisoning (Phenol Marasmus)		• General – anorexia, headache, vertigo, weight loss
		• Urine – dark
		• Pigmentation – yellowish (ocher like) discoloration of cartilages, sclera (oochronosis)

Biomarkers for Phenol Poisoning

Biomarkers are broadly defined as indicators signalling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility. Measurement of total phenol in the urine is the most useful biomarker following inhalation exposure to phenol (ACGIH 2001). The test is nonspecific and should not be used when workers are exposed to benzene, to household products, or to medications containing phenol. Dermal exposure may also result in overestimation of inhalation exposure. Dark urine occurs in individuals exposed to phenol. Phenol can also be measured in the urine after oral exposure, although a dose-response relationship between oral exposure to phenol and phenol in the urine has not been established. Specific biomarkers used to characterize effects caused by phenol have not been identified. The biological monitoring for exposure to phenol is possible by measuring blood or urine levels of the parent compound whose toxicity is suspected. The sample of urine to be tested can be stored in the refrigerator for 4 days or frozen for at least 3 months before analysis [11].

Diagnosis of Phenol Poisoning

Diagnosis of phenol poisoning can be made by

1. Typical odour.
2. Initial colourless urine that changes in colour to green or black.
3. To 10 ml urine add 1 ml of 10% ferric chloride. A purple or blue colour appears that persists even on heating.

Analytical Tests & Diagnosis (Phenols) [25, 26]

Qualitative Tests

- ***Iron (III) Chloride Test***

1. Iron (III) chloride test is used for water soluble phenols
2. 1 ml of distillate is taken in a clean and dry test tube.
3. 0.5 ml of water or water- alcohol mixture is added to it.
4. 1 to 2 drops of 1% aqueous iron (III) chloride solution is added to it.
5. A red, blue, green, or purple color formation indicates the presence of phenol.
6. The iron (III) chloride test for phenols is not completely reliable for acidic phenols.

- ***Iron (III) Chloride – Pyridine Test***

1. Iron (III)Chloride – Pyridine Test is used for water insoluble phenols.
2. 1 ml of distillate is taken in a clean and dry test tube.
3. 0.5ml of methylene chloride is added to it.
4. 3-5 drops of a 1% solution ferric chloride in methylene chloride is added to it
5. A drop of pyridine is added to it and stirred.
6. Addition of pyridine and stirring will produce a color if phenols or enols are present
7. This is more sensitive test for phenols.

- ***Quinonechloroimides Test***

Quinonechloroimides are slightly soluble in water. Quinonechloroimides decomposes slowly in alkaline buffers so the solutions to be tested should be very dilute, not stronger than 1 or 2 parts of phenol in 1000 parts of solution, should be brought to an alkalinity ranging between pH 8 to 10, preferable 9.4.2 or 3 drops of the test solution, carrying some of the quinonechloroimide in suspension, are added to 10 to 50 cc. portions of the solution to be tested. In the presence of reacting phenols the blue color of the indophenol develops, in the more concentrated solutions intense blue appears.

- ***Bromine water Test***

An unknown sample is treated with a small amount of elemental bromine in an organic solvent, such as dichloromethane or carbon tetrachloride. Presence of unsaturation and/or phenol in the sample is shown by disappearance of the deep brown coloration of bromine when it has reacted with the unknown sample. The formation of a brominated phenol in form of a white precipitate indicates that the presence of phenol. The more unsaturated an unknown is, the more bromine it reacts with, and the less colored the solution will appear.

Quantitative Test

The quantitative determination of phenol is done by UV-Visible spectrophotometer by making colour complex with suitable reagents and absorbance is measured at 610 nm. The color formation observed at a time intervals measured in minutes until the maximum of absorption is shown which requires from 10 to 20 minutes.

Management

Pre-Hospital Management [27]

Secondary contamination by victims exposed to phenol vapour is not a threat. However, victims whose skin or clothing are contaminated with liquid phenol can secondarily contaminate response personnel by direct contact or through off-gassing vapour from heavily soaked clothing or from vomitus. Hence, it is important that the clothes of the victim are removed as soon as possible. If inhalation exposure is there then the person should be removed from the site of exposure.

Hospital Management [4, 11-14, 16, 28]

- *Contact:* Remove clothes immediately. Clean skin with polyethyl glycol (PEG 400), isopropranol (70%), ethyl alcohol (10%), methylated spirit and olive oil. The use of water is dubious as in different studies varying results have been found. It is preferable not to use water for decontamination.
- *Ingestion:* Gastric lavage with water mixed with castor oil or olive oil or glycerine (10%), magnesium or sodium sulphate or saccharated lime or soap solution. When lavage is complete, 30 mg of magnesium sulphate or medicinal liquid

paraffin should be left in the stomach. Gastric lavage should not be done if esophageal injury is suspected.

- Demulcents, Egg white, Normal saline with sodium bi carbonate can be given.
- Increase hydration (glucose saline) to induce diuresis.
- Hemodialysis is not effective.
- On Inhalation - Patient should be removed from the contaminated area and given 100% humidified oxygen and ventilatory support.
- Cardiovascular support includes the use of intravenous saline and vasopressors to support the blood pressure. Lidocaine can be used to treat ventricular dysrhythmias and bretilium for lidocaine-refractory arrhythmias.
- Administration of sodium bicarbonate intravenously for central nervous system depression in the presence of metabolic acidosis.
- If methemoglobinemia > 30%, then administer methylene blue.
- Other supportive measure - artificial respiration, tracheal aspiration of froth/secretions

Post Mortem Appearance

Asphyxia (due to failure of respiration and edema of glottis and complications) and syncope[4]. Viscera should be preserved in saturated solution of sodium chloride and never in alcohol or rectified spirits. For histology, viscera should be preserved in formal saline solution [27].

Table 2: Post mortem appearance [4, 12-14, 16]

Post-mortem examination	Findings
External	• Smell – of phenol • Corrosion of skin • Tongue is white, swollen or hardened • Lips mouth throat – mucus membrane is coagulated, corrugated, detached, opaque, sdden and swollen, • Colour – whitened, brown or gray • Numerous small submucous haemorrhages are present.
Internal	• Esophagus- same as mouth • Stomach – contents – reddish fluid mixed with mucus and shreds of epithelium, smell of phenol. Thick and leathery mucosa. • Furrows are more damaged • Respiratory tract (inhalational phenol poisoning) - Coagulation necrosis of mucosa, sever congestion of submucous layers, laryngeal and pulmonary edema. • Liver, spleen – white hardened patch where stomach is in contact with them • Kidney – hemorrhagic nephritis • Brain – congested and edematous • Blood – dark, semifluid, partially coagulated.

Conclusion

Phenol is used in manufacture of many chemicals and substances. It is important to control the emission and release of phenol in surroundings to prevent air, water and soil pollution as it has been classified as an environmental pollutant and human health hazard. Though no record of carcinogenicity and bioaccumulation has been noted with phenol, it is still considered as extremely hazardous substance with reportable quantity (RQ) limit of 1000 pounds.[11] Periodical monitoring and adequate protective measure are required for prevention and early diagnosis of phenol poisoning. Medically, Phenol is often used as a disinfectant and preservative for vaccines and sera. Suicidal and Homicidal poisoning is rare because of taste and color of phenol, but accidental poisoning is due to carelessness in storage or misguided medical treatment like application to raw wounds or accidental overdose of phenol during treatment as analgesic [19] etc has often been reported.

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Forensic and Pharmacognostic Studies of Jatropha Curcas

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Abstract

Jatropha curcas is a multipurpose plant with many attributes and considerable potential. It is a tropical plant that can be grown in low or high rainfall areas and can be used to reclaim land, as a hedge or as a commercial crop. Now days the cases of Jatropha poisoning are rare, but due to its high toxic value in the presence of toxins like phorbol esters, the case studies in homicidal, suicidal or accidental cases become significant. For identification of *Jatropha curcas* constituents, an attempt has been made in the present study to identify phytochemicals, amino acid, saponins, phyto sterols, glycosides, anthraquinones, flavonoids and tanins. These were examined by various chemical colour tests and phorbol ester was examined by thin layer chromatography.

The extracted oil from the leaves and seed part was studied along with standard oil using two different solvents and their Rf values were also studied. For visualization of the spots, the plates were sprayed with sulphuric acid to develop coloured spots, which were then studied by UV in the range of 254-365 nm.

Keywords: Forensic; Pharmacognostic Study; Phyto-Chemicals; TLC; Phorbol Ester.

Introduction

Jatropha curcas (pinion of India) commonly called as 'Ratanjyot' in India is a drought resistant deciduous shrub which belongs to the family *Euphorbiaceae*. *Jatropha curcas* is native in tropical America, but is now found abundantly in many tropical and sub-tropical regions throughout Africa and Asia. Its strength as a crop comes from its ability to grow on very poor and dry sites. *Jatropha curcas* is a resilient plant that can adopt too many ecological conditions in any rural or urban areas of the country (Figure 1).

Due to the toxicity of its leaves, *Jatropha curcas* is not browsed and therefore traditionally used in protecting hedges around arable land and housing. The wonder plant produces seeds with an oil content of 37%. The oil can be combusted as fuel without being refined. Also due to its toxicity, *Jatropha curcas* oil is not edible and is traditionally used for manufacturing soap and medicinal applications.

Symptoms are largely those associated with gastro-intestinal irritation. There is acute abdominal pain and a burning sensation in the throat about half an hour after ingestion of the seeds followed by nausea, vomiting and profuse watery diarrhea. In severe poisoning, these symptoms progress to hemorrhagic gastroenteritis and dehydration. Polydipsia can be extreme. Salivation and sweating may occur. There may be skeletal muscle spasm. Intense hyperpnoea or a quick panting respiration is seen together with hypotension and electro cardio graphic abnormalities. There may be CNS and cardiovascular

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depression, children are more susceptible. This may be either a direct effect of toxins or secondary to dehydration.

Fig. 1: *Jatropha curcas Plant*

The toxicity of *Jatropha curcas* is based on several components (phorbol esters, curcains, trypsin inhibitors and others). There are possibilities of its consumption knowingly or accidentally. Due to its high toxic value, mainly in presence of toxins like phorbol esters, the case studies in homicidal, suicidal or accidental cases become significant.

Phytochemicals of Jatropha Curcus

The following phytochemicals have been detected in *Jatropha curcus*

Curcin

Curcin is a toxic albumin belonging to a group of proteins called ribosome-inactivating proteins (RIP), which inhibit prokaryotic and eukaryotic ribosome by specific modification of the larger rRNA. Based on physical properties, RIPs are classified into-

- *Type 1 RIP* which are single-chain (approximately 30 kD) proteins having enzymatic activity and inhibiting cell-free protein synthesis *in vitro*, but are relatively non-toxic to cells and animals.
- *Type 2 RIP* which are heterodimeric proteins (approximately 60 kD) consisting of A chain (similar to type 1 RIP) attached to sugar-binding B chain (lectin) by a disulfide bond. Type 2RIP is highly toxic compared to type 1 RIP, which are relatively nontoxic *in vivo* due to the absence of the sugar-binding chain.

Diterpenes (Phorbol Esters)

The term 'phorbol esters' is used today to describe naturally occurring group of compounds mainly distributed in plant species of the Euphorbiaceae family. Phorbol esters are esters of phorbol, a tetra cyclic diterpenoid with a tigliane skeletal structure. Terpenoids are classified according to the number of carbon atoms they carry. Monoterpenoids have 10 carbons; diterpenoids have 20, and so on. (Fig 2)

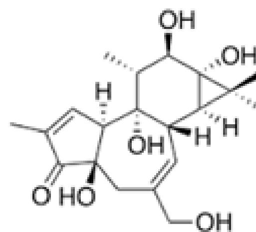

Fig. 2: Phorbol esters

Diterpenes are believed to be the most potent compounds synthesized by *Jatropha* species. There are at least 20 diterpenes reported from *Jatropha* species. Among the diterpenes, a group of compounds having tigliane skeleton called phorbol esters are the most toxic molecules in *Jatropha* species. Six phorbol esters are identified in *Jatropha curcas*. The concentration of phorbol esters varies from 2 to 3 mg/g kernel meal and from 2 to 4 mg/g oil in different provenances of *Jatropha curcas*. The phorbol esters are lipophilic, present mainly in oil, and when present in oil or kernel not affected by heat. In *Jatropha curcas* the phorbol esters (mg/g dry matter) are present in kernels, leaves, stems flowers, buds, roots, bark(outer brown skin) bark(inner green skin) and wood, but not in latex.

Other Compounds

Tannins

Tannins are the phenolic substances associated with toxic and impaired nutrient absorption and anti-nutritional effects including reduced food/feed intake, growth retardation. Tannins possess multiple phenolic hydroxyl groups leading to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids, and polysaccharides.

Saponins

These are steroid or triterpene glycoside compounds which are present in a variety of plants.

In plants, saponins may serve as anti-feed ants or help in protecting the plant against microbes and fungi. However, saponins are often bitter in taste, and thus, when present in high concentrations would reduce plant palatability in livestock.

Phytates

Phytic acid (known as inositol hexakisphosphate IP_6 or phytate when in the salt form) is the principle storage form of phosphorus in most plant seeds. Inositol penta- (IP_5), tetra-(IP_4), and triphosphate (IP_3) are also termed phytates. Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because these animals lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule. Phytates also form sparingly digestible phytate–protein complexes, thus reducing the availability of dietary protein.

Lectins

Lectins are carbohydrate-binding (glyco) proteins and are ubiquitous in nature. Plant lectins when consumed by animals survive digestion in the Gastro Intestinal Tract and bind to membrane glycosyl groups of the cells lining.

Experimental Procedure

Materials

The samples were collected from the wild patch of *Jatropha curcas* plants grown at the Ajmer city of Rajasthan (N: 26° 26' 59.6256'', E: 74° 38' 23.6940'') in the month of February. Fresh leaves and seeds has been collected from the same plants and stored at 4°C in aluminium foil.

Methods

Macroscopic and Microscopic Study

Fig. 3: *Jatropha curcas Leaf*

Macroscopic Examination

Leaves: *Jathropha curcas* has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The leaves are arranged alternately with apetiole 3 to 20 cm long and broadly ovate in outline (Figure 3).

Seeds- Seeds of *Jatropha curcas* are ellipsoid, 1 to 2 cm long, mottled black and coarsely pitted. The seeds become mature when the capsules change from green to yellow after two to four months. *Jatropha curcas* growth rate is variable and produce seeds after approximately two years depending upon many factors as rainfall, etc. Three months after sowing, mean height growth of seedlings produced in greenhouse conditions ranged between 15 cm and 33 cm and showed variability among seeds. The seed yield up to 31-37% of valuable oil (Figure 4).

Fig. 4: *Jatropha curcas Seeds*

Microscopic Examination

Fresh leaves of *Jatropha curcas* were considered for the microscopic examination. For the purpose trinocular microscope was used. Different sectional views were taken from surface view of epidermis and lamina (Table 1).

Table 1: Characteristics of jatropha curcas leaf

Properties	Observation
Colour	Dark Green and Light Green
Shape	Broadly Ovate
Size	10-15 cm. x 7.5-12.5 cm
Apex	Acute
Venation	Palmate
Margin	Cordate
Taste	Characteristic, Bitter

In microscopic examination of the fresh leaf, midrib showed 5-7 layered thick walls, closely packed collenchymatous cells on both the surfaces with spongy and vascular bundles. Upper epidermis was covered by thin cuticle. Both the epidermis showed anomocytic stomata. Simple covering trichomes were very rare on both epidermises. Transverse view of lamina showed single layer of closely packed

palisade cells below the upper epidermis layer of the leaf (Figure 5).

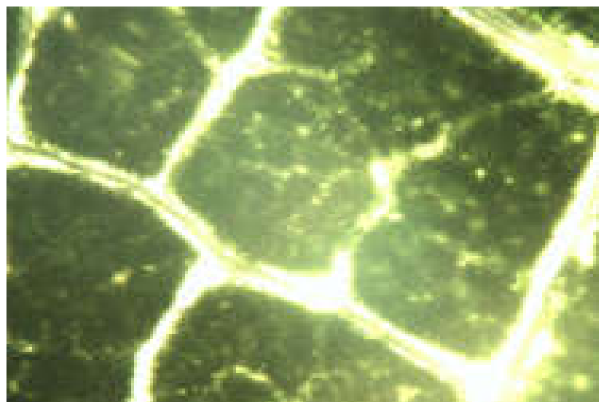

Fig. 5: Microscopic view of leaf

Qualitative Screening of Phytochemicals

N-hexane and butanol extract of leaves of *Jatropha curcas* were screened for the presence of alkaloids, amino acids, anthraquinones, flavonoids, glycosides, phytosterol and saponins by using standard protocols.

Preparation of the Extract

Jatropha curcas leaves fully dried at room temperature were grinded into powder and for it, ten grams was weighted accurately and extracted with n-hexane in a Soxhlet extraction apparatus. At the end of the extraction process, the flask containing n-hexane extract was removed and n-hexane was evaporated by using a rotary evaporator. The weight of the residual extract was measured and precent yield was calculated. The same procedure was used to prepare butanol extract. These extracts were kept in refrigerator for phytochemical study.

Extract yield% = $W_1/W_2 \times 100$

Where W_1 = net wt. of powder in grams after extraction.

W_2 = net wt. of powder in grams taken for extraction.

Screening Test

One gram of ethyl acetate, acetone: water (7: 3) was dissolved in 100 ml of its own mother solvents (in n-hexane as well as in butanol extract) to obtain a stock of concentration 1% (V/V) for the screening test.

Chemical tests were carried out on the extracts using standard procedures to identify the constituents by characteristic colour changes as described by Sofowara (1993), Obebedy and Sofowara (1978) (Table 2).

Test for Alkaloids

In 5ml stock extract 2 ml HCl was added. It made acidic medium and then 1ml of dragendroff's reagent was added, which gave orange or red color precipitate. It indicated the presence of alkaloids.

Test for Amino Acid

In 2 ml stock extract 40% NaOH solution was added in a test tube. After that one drop of 1% $CuSO_4$ solution was added in it. A blue color appeared which showed the presence of amino acids in the extract.

Test for Anthraquinones

2M HCl was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then it was cooled and filtered. The filtrate was extracted with chloroform layer which was later on separated and shaken with 10% KOH and it became pink-red which showed the presence of anthraquinones in the extract.

Test for Flavonoids

Dilute NaOH (1N) was added to one ml extract, then yellow color in plant extract appeared, and soon it became colorless, when few drops of acid (10% H_2SO_4) were added to it. It indicated the presence of flavonoids in the extract.

Test for Glycosides

On water bath, 1ml of extract hydrolysed with HCl for few hours and cooled at room temperature. Then to it 1 ml pyridine was added with a few drops of sodium nitropruside solution, which further made alkaline with NaOH solution. Pink to red color appeared, which indicated the presence of glycosides in the extract.

Test for Phytosterol

The extract was refluxed with solution of alcoholic KOH till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for presence of phytosterol. The residue was dissolved in few drops of dilute acidic acid; 3ml acetic anhydride was added followed by few drops of conc. H_2SO_4. Bluish green color appearance

indicated the presence of phytosterol in the extract.

Test for Saponins

Extract was diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes continuously. A formation of layer (1cm) of foam showed the presence of saponins in the extract.

Table 2: Phyto-chemical analysis

S. No.	Chemical Tests	N-hexane Extract	Butanol Extract
1.	Alkaloids	+	+
2.	Amino acid	-	+
3.	Anthraquinones	-	-
4.	Flavonoids	+	-
5.	Glycosides	-	-
6.	Phytosterol	-	+
7.	Saponins	-	+

(Note: +=present, -=absent)

Identification of Phorbol Ester by TLC

A standard glass TLC plate was coated with the slurry of silica gel G in water to a uniform thickness of 0.25 mm. After that the plate was activated by heating in an oven at 110^{0}C for about one hour. The extracted material is then spotted on silica gel G spotting plate with the help of micro capillary tube, and then transferred to the saturated chromatographic chamber in which appropriate amount of solvent was added and allowed to developed till the solvent front move up to 7 cm with the developing solvent to a fixed demarcated finishing line and then plates were taken out and allowed to dry in open. The whole process was repeated for two different solvent systems. For visualization of the spots, the plates were sprayed with sulphuric acid to develop coloured spots, which were then studied by UV in the range of 254-365 nm (Figure 6).

The rf value of phorbol esters in hexane: ethyl acetate: glacial acetic acid (30:60:10) was 0.39 (reported standard 0.40) whereas in dichloromethane: acetone (90:10) was 0.79 (reported standard 0.80).

Fig. 6: TLC photographs showing the movement of extracted compound (left side) from spotting point with the standard compound (right side) in each TLC plate when viewed under UV chamber

Results and Discussion

In the present study an attempt has been made to study *Jatropha curcas* plant by Macro and Microscopic, Phytochemical and TLC examination. These examinations are found to be very useful tools for the identification and characterization of *Jatropha curcas* leaves and seeds. A simple, accurate and precise analytical method is used for the analysis of leaves and seeds of *Jatropha curcas*, which could be useful in future forensic identification of unknown plant material. It has been observed that Macro and Microscopic, Phytochemical and TLC examination are very useful tools for the identification of *Jatropha curcas* leaves and seeds. Phytochemical studies were carried out for the identification of *Jatropha* leaves with standard plant leaves. Thin layer chromatography studies showed the presence of active principles of *Jatropha curcas*. This is further suggested that the proposed methods are simple, sensitive, reproducible, and economical and requires very less equipment. These can be employed for qualitative evaluation of *Jatropha curcas* leaves and also for the routine forensic analysis of *Jatropha curcas*. Therefore, this could be a method of choice for official monographs in Forensic Toxicology.

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Doctor's Witness and Court Procedures in India

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Abstract

Law always need the help of medical expert in one way or other for justice as it has no such expertise. Sometimes, the doctor is the only material witness available. So, the court calls a doctor for help whenever it feels necessary. To attend a court is not less than a great ordeal for a doctor, as he is not well verse with the functioning of the court. This article is to introduce doctors to the court procedure so that he does not feel estrange whenever he had to face it.

Keyword: Summons; Medical Expert; Legal System; Court; Evidence; Witness; Conduct Money; Hostile Witness.

Introduction

The court has no medical expertise and not expected to have. It is dependent on a doctor for any opinion regarding the medical aspect of the fact at hand. Doctor can be of help as an expert in presenting the unbiased view of various events and in corroborating the facts presented by either party in a dispute. Sometimes, medical evidence is the only evidence available to the court when no eyewitness is available or come forward.

Giving testimony in the court of law is a responsible task. Most of the doctors try to avoid getting involved in it. There are various reasons behind it, but most importantly, time involvement and merciless cross-examination. This is mainly because doctors are not acquainted with the court and court procedure. This article is to provide some outline about how court usually interacts with a doctor as an expert witness and how a doctor should interacts with the court.

Summon

There are various processes to compel the appearance of an accused or any witness, expert or common, to give evidence in the court of law like Summons, Warrant of Arrest and Proclamation & Attachment.

Summon or Subpoena [1] (Latin, sub=under, poena=penalty) is a document compelling the attendance of a witness in a court of law under penalty, on a particular day, time and place, for the purpose of giving evidence.The court may also ask him to bring various books, documents or any other things under his control to produce on the court as evidence.

Section 311 of the Code of Criminal Procedure, 1973 deals with the power to summon material witness, or examine person present. Any Court may, at any stage of any inquiry, trial or other proceeding under this Code, summon any person as a witness, or examine any person in attendance, though not summoned as a witness, or recall and re- examine

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any person already examined; and the Court shall summon and examine or recall and re- examine any such person if his evidence appears to it to be essential to the just decision of the case.

Section 61 to 69 CrPC deals with summons. A summon is issued by the court in duplicate signed by the presiding officer and bears the seal of the court. It contains the name of the accused and defendant, FIR number, the various dealing IPCs, police station with which the FIR registered, date, time and place of hearing and brief account about the role of the witness in the particular case, including the relevant document name and number like postmortem report or /medico-legal report.

Subpoena/Summons are of Two Types [2]

- Subpoena ad testificandum: To attend and give evidence.
- Subpoena duces tecum: To attend, bring documents as specified and give evidence.

Serving of Summon [1,3,4]

Summon is served to the person by a police officer, officer of the court or any other government servant. The person keeps one copy giving receipt on the other copy. Summon can be served by the registered post. If the person to whom summon to be served is not found, summon can be served to any adult person of the family residing with him after taking receipt or can be fixed on the house where the person resides. If the person is in active government service, summon is usually sent in duplicate to the head of the office and such head will ensure delivery of summons to the particular person. When a Court issues summon to be served at any place outside its local jurisdiction, it usually sends such summons in duplicate to a Magistrate within whose jurisdiction where the person summoned, resides.

Under the code of civil procedure, a summon can be e-mailed or faxed by the court.

If a person refuses to accept summon, summon can be fixed on the house or workplace or court can issue arrest warrants to ensure his attendance.

Attending a Summon [1,4]

A summon must be obeyed and all the evidences must be submitted, as asked by the court. The person can be excused from attending the court if he has a valid reason but the reason for not attending the court should be intimated to the court in advance to avoid complications or penalty. The court generally accepts the genuine reasons like on health ground and allows to appear on the next date.

Whenever there are more than one summon at a particular time to attend, the witness should know that the criminal court has priority over civil court and he should attend the criminal court duly informing the civil court. In the same way, a higher court has priority over lower court. If summon is from the court of the same status, the witness should attend the court from which he got summonedearlier, duly informing the other court. The witness can attend the second court after finishing his evidence in the first court.

Non-Compliance of the Summon

When a witness fails to attend the court on the date of summon, the court doesn't straight forward issue notice/warrant to the witness. The court will ask the 'proof of service' i.e. the return receipt of certified mail, signature of the person receiving summon or the person who served summon swears that summon was served. In the absence of 'proof of service' the court cannot issue notice/warrant because you were not duly served. A telephone call or a faxed/emailed summon will not constitute 'proof of service' but if he appears to the 'electronic summon' it will be taken as 'proof of service'.

If the witness fails to attend the court intentionally without any valid reason, then he will be liable to pay damages in civil cases. In criminal cases, the court may issue notice (Sec 350 CrPC) and after hearing the witness, if the court finds out that the person neglected summon without any valid reason, may sentence fine on him or imprisonment or may issue bailable/non-bailable warrant to secure the attendance of the witness. Generally the court issues a bailable warrant, followed by non-bailable warrant. On rare occasion, the court may penalize the person by making him stand 'till the rising of the court'. Sometimes the court can bind the witness for the next appearance with his consent in the court itself without the need for issuing next summon. Then, he is bound to appear on the next date.

Nonattendance of the court in response to a court order without any valid reason may cause imprisonment up to six months or fine upto 1000 rupees or both (Sec 174 IPC).

Expenses to Attend the Court [1,5,6,7]

In civil cases, a fee is paid to the witness at the time of serving summons to cover the expenses of attending the court known as conduct money.

Conduct money is legally defined as 'money paid to a witness who has been subpoenaed on a trial, sufficient to defray the reasonable expenses of going to, staying at, and returning from the place of trial'. If the witness thinks that the fee is not sufficient, he can bring this fact to the notice of the court before giving the evidence.

In criminal cases, no fee is paid to the witness at the time of serving the summons. But the witness should attend the court and give evidence in the interest of the state. Conveyance charges and daily allowance can be claimed by the witness, according to the government rule. Nowadays, in all cases in which an officer of Government is summoned to give evidence, the Court gives him a certificate in the prescribed form, specifying the dates on which the officer attended the court and the amount, if any, paid to him by the Court. This certificate can be used by the officer concerned for claiming the travelling allowance from his employer, if he was not paid by the court. The certificate of attendance is provided in both civil and criminal cases.

Legal Procedures in India [1,4]

The witness is defined as 'a person who can give evidence regarding facts and/or the inferences that can be drawn therefrom' [1,4] (Sec 118-134 IEA). Legally,a witness is a person whose declaration under oath (or affirmation) is received as evidence for any purpose, whether such declaration is made on oral examination or by deposition or affidavit [16].

Witnesses are of two types: common witness and expert witness.

Common Witness is witness of fact who can give evidence about the facts observed or perceived by him.

Expert Witness (Sec 45 IEA) is a person who is trained, skilled or has education, knowledge or experience in a technical or scientific subject and capable of drawing inferences/ opinion from the facts observed by him or by others, as doctors, firearm experts, fingerprint experts, handwriting experts, etc.

An expert may give an opinion upon facts which are either admitted, or proved by him or other witnesses at the trial; on matters of common knowledge; and on hypothetical questions based on assumptions. The main obligation of a doctor is to bring professional facts. A doctor's testimony is considered evidence only when he can prove that his inferences are based on reasonable medical certainty (more probable than not) that a fact is true or not. Conclusions must be based on facts.

Experts are of two types: [11]

• Non-testifying Expert:

– Hired by a contesting party to evaluate the facts of the case. The expert helps the attorney to prepare a case, without testifying in the court

• Testifying Expert

– The expert appears in the court to testify before the judge, under oath

A witness who is supposed to have some motive or interest in concealing part of the truth or giving false evidence is declared as a hostile witness by the court on the suggestion of the lawyer of the summoned party. Hostile witness or adverse witness is defined as a witness who gives negative evidence against the party who called them as a witness [9].

In a significant order in the sensational Naga Vaishnavi murder case, the Andhra Pradesh high court stated that no expert witness could be treated as hostile by the prosecution. The HC bench headed by Justice Raja Elangovan said that the forensic expert was only an expert from outside the prosecution and their witness could not be considered as prosecution witness. He, however, said that the prosecution could cross-examine the forensic expert if they wanted to without treating him as hostile [12].

Hostile witnesses can be charged with perjury, which means giving wilful false or fabricated evidence (Sec 191 IPC) and may be prosecuted with imprisonment up to seven years (Sec 193 IPC).

Doctor as a Witness

A doctor can be both a common and expertwitness. When he describes injuries present over the body, he is a common witness and when he draws inferences and opinion about the inflicting weapon and the manner of infliction, etc., he is an expert witness.

As per Section 45 of Indian Evidence Act, the opinion of an expert is admissible, as opinion on a scientific fact by a person skilled in that subject is a relevant fact. However, the opinion of an expert is not binding on the court, as the expert is not a witness of facts and his evidence is of an advisory nature. He needs to put all the material facts before the court and the reasons on which he concludes the opinion, so that the court can make his own judgement.

Medical evidence is only corroborative evidence. It is of little value when there is a conflict of opinion between experts. In that case, Courts usually accept the opinion which is not in conflict with the direct evidence. But if direct evidence is not trustworthy, conviction may rely on medical evidence, if it is

trustworthy.

Recording of Evidence [1,4]

When the doctor appears before the court, the court will generally record their evidence promptly and as far as possible, they will not be required to attend the court at any adjourn hearing. The court will provide a tentative hour when the evidence is likely to be recorded. There is a standing order by Delhi High Court that the doctors should be summoned after 2 pm, so that it does not affect their patient care.

Evidence is recorded after taking oath. There are three main steps in recording evidence: Examination-in-chief by the counsel for the party who called him/her, Cross-examination by the opposing counsel and Re-examination by the prosecution counsel. Judge can ask any question at any stage to clear any doubt.

Examination-in-Chief

It is the first examination of the witness. Examination-in-chief is done by the lawyer for the party who called the witness. In a criminal case,the state is the party and the doctor is first examined by the prosecution lawyer.The object of this examination is to elicit principle salient facts bearing on the case, all relevant, medical facts and the conclusion that has been drawn from the facts. At this stage no leading question allowed except when the presiding officer is satisfied that the witness has turned hostile.

The Doctor should help the prosecutor in framing proper questions in proper sequence, so that each and every essential fact can be elicited.

Cross-Examination

In this the witness is examined by the lawyer of opposite party i.e. defence lawyer. The main objective of this examination is to elicit facts which are favourable to his case, to test the accuracy of the statement given by the witness during chief examination and sometimes to discredit the witness. Competency and credibility of the witness are also questioned.

The doctor should keep in mind that the facts favourable to the defence side should be given as promptly as on chief examination. Any omission should be accepted as the entire test given in a treatise for a single diagnosis need not to be done, which can be explained to the court. The doctor should not be dogmatic about his opinion.

Cross-examination helps to test the reliability of the evidence given. The object of cross-examination is to weaken, qualify or destroy the case of opponent. When the defence starts humiliating or embarrassing the witness, he may become tense, frightened, angry, aggressive or hostile. He should face the cross-examination coolly and intelligently, and should on no account lose his temper. Any self-incriminating statement given during cross-examination doesn't make him liable for arrest or prosecution afterward.Leading questions are allowed during cross-examination. It has no time limit.

Re-Examination

This is conducted by the first lawyer. The object is to correct, clarify or add any details to the statement the witness had made during cross-examination.It is allowed only when the presiding officer allows thinking it proper, but still leading question is not allowed.

Question by Judge

The judge may ask any question, in any form, about any fact, irrelevant or relevant, at any stage of examination to clear any doubts which arise. The court has the power to examine the witness even in the absence of public prosecutor, so that the person behind the scene doesn't suffer [13].

The deposition of the doctor is written and handed over to him. The witness should go through all the papers and sign all the papers. The witness should leave the court only after due permission of the court. The court can recall and re-examine any witness who is already examined if the court think it essential to examine the witness again.

In Witness Box

Some rules which can help a doctor in the witness box in avoiding unnecessary embarrassment are as follows:

A doctor should attend the court in time with all the required documents as asked and well prepared, and if necessary study the literature on the subject. He should appear professional and should not talk loosely about the case to anyone. As per Bouardel: if the law had made a physician a witness, he should remain a man of science; he has no victim to avenge, no guilty person to convict and no innocent person to save.

A doctor should be impartial. Any fact or opinion which is favourable to the defence should be given as promptly as to the prosecution. His evidence should be within the limit and scope of science and

his field of expertise. He should avoid speaking on a subject in which he has little or no practical experience. Irrelevant questions can be brought to the notice of public prosecutor/court.

The most important thing is how the doctor clarifies the facts and opinions to the court. He should speak coolly and calmly with full confidence, should use simple words and avoid technical terms. Under any circumstances, he should not lose temper. An angry witness is often a poor witness, and the effectiveness of his testimony is diminished or destroyed.

He should answer briefly and directly to the question asked by the counsel or the court, but if any question requires an explanation, the doctor should resists to give simple answer and explain it. If the question is not understood, he can politely ask to clarify or rephrase it. If any question is not known to him or beyond his expertise, he should admit it without any hesitation. A doctor has no professional privilege, and therefore he must answer any question, but only if the court directs to do so.

A doctor can volunteer things to the court, if he thinks necessary to avoid any confusion or misleading which may lead to miscarriage of justice. But, information should not be volunteered beyond that asked for in the question as it is often not well prepared, and is liable to cross-examination.

There are Some Guidelines Proposed by the General Medical Council, UK, for Medical Witnesses [10]:

1. The role of an expert witness is to assist the court on some special or technical matters which are within their expertise. His duty to the court overrides any obligationto the party who is paying him. So, the expert has to be independent and not influenced by the party calling him.
2. He must understand the question asked before giving any answer. He should not give any answer/opinion until he understands the exact question.
3. He must restrict his view/opinion/statement to the area he has relevant and direct experience. He must be aware of the standard of various procedures at the time of the incidence.
4. If any issue/question falls outside his expertise, he should make it clear to the court. If the court still ordered to answer, he must answer with his best of abilities, making it clear that the issue is outside of his competency.
5. His opinion must be balanced and he must clear the facts or assumption on which the opinion is based on.
6. All the reports written or evidence given should be accurate and must not be misleading.
7. If he is asked to give advice or opinion about any person without consultation he must express the limitation.
8. His language and terminology should be readily understood by the non-doctor. He must explain any medical terminology or abbreviation used.
9. If at any stage the medical witness changes his view on any material/matter, he must convey the fact to the calling party, opposite party and to the court. Usually, the expert informs the calling party counsel and he ensures to inform the opposite counsel and the court.
10. The most important thing is to be honest, trustworthy, objective and impartial. He should not be influenced by the facts and individuals related to the proceedings.
11. He must keep upto date in his field. He must understand the law of the land related to his work. He also should know how to prepare a court-compliant report and how to give oral evidence.
12. If proper consent has not been obtained for disclosure of the information from the concerned party, he should inform the court. He should not disclose the confidential information other than to the party to the proceedings, unless the subject consents or obliged by law to do so or ordered by a court or tribunal to do so.
13. If there is any potential conflict of interest of the expert or personal interest, he must remain impartial. He may continue as an expert witness only when the court allows.

Statute of the Expert Witness in Indian Senario [14]

Scientific evidences are accepted by the court under Sec 45 IEA as there is no specific rule or law related to this as opposed to various rules prevalent in American law, e.g. 'The Frye Test', 'The Federal Rule of Evidence' and ' The Daubert Guidelines'.

According to the section 45 IEA 'When the court has to form an opinion upon a point of foreign law, or science or art, or as to the identity of handwriting (or finger impressions), the opinions upon that point of persons specially skilled in such foreign law, science or art, (or in questions as to the identity of handwriting or finger impressions) are the relevant facts.

According to the courts, medical evidence is only

an opinion and it is hardly decisive. But the opinion of the autopsy surgeon and of the forensic science laboratory is reliable. The Supreme Court has stated that unless there is something inherently defective in the medical report, the Court cannot substitute its own opinion for that of the doctor. The court is unlikely to understand the various principles and methods of the procedure, so the opinion of experts is taken by the court solely on trust and faith.

According to The Supreme Court Of India 'A medical witness called in as an expert and the evidence given by the medical officer is really of an advisory character based on the symptoms found on examination. The expert witness is expected to put before the court all materials inclusive of the date which induced him to come the conclusion and enlighten the Court on the technical aspects of the case by explaining the terms of science, so that the Court, although not an expert, may form its own judgement on these materials after giving due regard to the expert's opinion because once the expert's opinion is accepted it is not the opinion of the medical officer but that of the Court.

Witness Protection [15]

In current scenario, India does not have any specific law or act to protect important witness as in USA, where there is the Federal Witness Protection Program and in Canada, Witness Protection Act which provides protection to its witnesses. But, there are guidelines/orders issued by various courts for the protection of important witnesses and their relatives.

Delhi High Court has provided some guidelines for the protection of witnesses. The Court has also made it compulsory for the investigating officer of a case to inform the witness about the new guidelines. The Court has appointed the Member Secretary of the Delhi Legal Services Authority to decide whether a witness requires police protection or not. The competent authority shall take into account the nature of security risk to him/her from the accused, while granting permission to protect the witness. Once the permission is granted, it shall be the duty of the Commissioner of Police to give protection to the witness.

Besides this, Mumbai police has formulated four-point plan to protect the vital witnesses in the bomb blast and other sensitive cases. The plan is based on the following guidelines:

1. Transferring the witness from his city of residence to another city.
2. The government will provide the witness with a job similar to the one he is/was doing.
3. The witness shall be given a new name, identification, ration card; and a new passport.
4. The government will accept the responsibility of the witness's entire family and provide it with security cover.

Under present circumstances, the Indian Government is evaluating the American laws pertaining to witness protection, where gang men after turning approver are given a new name and identity and relocated to a new place.

Recording via Video Conference [16]

Indian statutes do not have specific provisions for recording of evidence via video conference, though courts through various landmarked decisions laid down various parameters and framework for the use of video conference and internet conference.

This has been consolidated in the landmark decision of the State of Maharashtra v Dr Praful B Desai in 2003. In the case Supreme Court upheld video conferencing as a vital tool for recording of evidence, particularly for the witnesses residing abroad.

Firstly, it was used in civil proceedings in cases where the witness is residing abroad or cannot attend the court for various reasons. Courts observed that it saves time and expenses and also helps in rapid disposal of cases.

The courtalso has used the technology on several other occasions depending upon the facts and circumstances, mainlyan examination of sexually exploited victims.

In the case of Dr Kumar Saha v Dr Sukumar Mukherjee in 2011, the Supreme Court allowed recording of testimonies and cross-examination through internet conferencing.

Guidelines for Video Conferencing

Certain guidelines are laid down by the judiciary for proper identification of the witness and accuracy of the equipment. They can be summarised as follows:

1. All the expenses and arrangements for video conferencing have to be borne by the applicant, who wants to avail the facility.
2. An officer will be deputed, from India or from the consulate/embassy inthe country where the evidence is being recorded, who will have some important responsibility. He would remain

present during the process and willensure that there is no other person in the room while the evidenceis being recorded. He will fix the time for recording evidence.

3. The evidence will be recorded during working hours of Indian courts. No plea of anyinconvenience will be accepted on account of the time difference between India and the concerned country where the witness is.
4. If the witness doesn't attend at fixed time, without any sufficientcause, then the Magistrate may disallow recording of evidence by video conferencing or may take action asprovided by law, to compel attendance.
5. In case of non-Party witnesses, all the relevant and disclosed documents will be sent to the witness for theiracquaintance and an acknowledgement will be filed.
6. The visual will be recorded at both ends.
7. The witness would have to filean affidavit/ undertaking duly verified before a judge/ magistrate/notary that the person shown asa witness is the same person as who is going to depose with a copy of such affidavit to the otherside, before the process of evidence recording starts.After identification process, oath would be administered as per the Oaths Act 1969 of India, by an officer duly authorised for that.
8. The person who wishes to examine such witness would have to file anaffidavit/undertaking.
9. The officer deputed will ensure that the witness is not coached/tutored/prompted. He will ensure that the witness, their counsel and one assistant are allowed in the studio during the process. He will also ensure that the witness is notany way prevented from bringing into the studio any papers/documents which may be required. The witness alone can be present at thetime of the process of evidence recording.
10. Magistrate and notary are to certify to this effect.
11. The officer deputed will ensure that the whole process proceeds without any interruption and without any adjournments.
12. The officer will take memo if the witness doesn't reply to any answer and this will be taken into consideration while the evidence is being read in the court.
13. The court/commissioner must record any remark regarding the demur ofthe witness while on the screen and shall note the objections raised during the recording of thewitness either manually or mechanically.
14. To become part of the record of the proceedings, the depositions of the witnesswill have to be signed by the witness as early as possible before a magistrate or notary public. The digital signature can be adopted in thisprocess and will be obtained immediately after day's deposition.

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