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CHEMICAL ANALYSIS OF ENDOSULPHAN

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Abstract

Endosulfan is an easily available insecticide and mainly used for agricultural purposes. Hence most of the villagers use endosulfan for committing suicide. The recent cases reported in toxicological laboratories show that suicide with endosulfan is increasing in these days. Endosulfan causes most degree of death because of its toxic effects. The toxicology division of forensic science laboratory plays vital role in analysis of poisons through Criminal Justice System. In every death case which is connected to the criminal justice system, the human viscera of deceased are sent to forensic sciences department for analysis. In a case report, the toxicology division received human viscera of a deceased from the forensic medicine department where a person was declared death after consuming endosulfan due to severe stomach pain. It was brought to toxicology division of forensic science laboratory where the type of poison was identified and estimated. Two methods such as thin layer chromatography and UV Visible spectrophotometry were adopted to analyse the human viscera. The forensic analysis includes solvent extraction, identification and estimation.

Keywords: Analysis, endosulfan, forensic, thin layer chromatography, UV visible

INTRODUCTION

The application of forensic science in criminal justice system is multiple ways. In order to utilize the forensic science, the toxicology takes a vital role in identification of intoxication in legal cases. The laboratory work in this division determines types of drugs/pesticides, quantity, its concentration and it gives a clear way to determine whether the quantity of drugs or pesticides alone lead to one's death or not.[1-4] This work describe about human viscera which was brought to toxicology division of forensic science department through legal system. In a case report, it was found that a farmer committed suicide due to severe stomach pain. The farmers used these insecticides to destroy the insects in the field.[5,6] The organochlorine (chlorinated hydrocarbon) insecticides are a diverse group of agents belonging to three distinct chemical classes. These agents were used extensively in all aspects of agriculture and forestry, in building and structural protection and in human situations to control a wide variety of pests.[7]

Endosulfan is one of the most widely used spectrum cyclodieneorganochloric insecticide and highly toxic endocrine disrupter and a contact stomach poison.[8-10] The neurotoxic endosulfan is registered under Federal Insecticide Fungicide and Rodenticide Act by the United States Environmental Protection Agency (USEPA).[11] Chemically it is named as 6,7,8,9,10,10- hexa chloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide.[12] Endosulfan is acutely toxic and has been implicated in many cases of poisoning and fatalities. It has been identified with a range of chronic effects, including cancer and impacts on hormonal systems, exhibiting similarities with its predecessors in the organochlorine class.[13-15] Cases of endosulfan poisoning have been reported from many parts of the world. Accidental and intentional exposure leading to human fatalities and environmental tragedies has occurred.[16-17]

Paul and Balasubramaniam have studied the effect of single and repeated administration of endosulfan on behavior and its interaction with centrally acting drugs in experimental animals.[18] Rao D.M, and A.S. Murthy have conducted the experiment on persistence of endosulfan insoils.[19] According to Sinha, Narayan and Saxena, endosulfan induced biochemical changes in testis of rats.[20] Miles and Moy analysed degradation of endosulfan and its metabolites by a mixed culture or soil microorganism.[21] Jain et al. and Oraket al conducted a case study of endosulfan poisoning and found that endosulfan poisoning lead to consciousness, vomiting and seizure. If there is a sudden rise in body temperature, stiffness in limbs or massater spasm occurs in the case of endosulfan poisoning. [22-24] Some of the studies reported that the nervous system get collapsed due the effect of endosulfan. [25-26]

Alva et al proved that exposure to endosulfan results in a significant rearrangement in the biochemical parameters with a decrease in antioxidant levels in the heart and liver. This is an early indication of pathogenesis in the vital organs of rats.[27] Singh et al, found that apoptosis played a significant role in the pathogenesis of endosulfan and citrinin toxicity.[28] Gude and Bansal reported that endosulfan poisoning is complicated by multi-organ dysfunction, cardiac arrest and death.[29] Sharma et al., found that uncommon presentation of endosulfan poisoning in a 32 year old male with high anion gap severe refractory metabolic acidosis.[30] In the present study, we report a suicide case of farmer, who consumed endosulfan. The endosulfan consumed was identified in viscera, using various spot tests and thin layer chromatography. The quantity of poison consumed was estimated using UV-Visible spectrophotometric method.

METHODS

The endosulfan was identified by treating the extract with Nickel-amine and o-tolidine reagents. Endosulfan was identified when the formation of greyish-black spots appears by spraying the developed plate with 20% sodium hydroxide solution followed by nickel amine reagent. It was also confirmed through the conversion of black spots into blue by spraying on the same plate o-tolidine reagent. The solvent system is hexane and acetone in 9:1 ratio. In another method, bluish-green colored spot was formed on spraying the

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developed plate with o-tolidine reagent followed by exposure to UV irradiation or sun rays for 10 min. in same solvent system used as similar to earlier experiment.

In the experimental part, viscera were divided into three portions viz., stomach, intestine, liver and kidney. One tenth of each portion was taken in a conical flask. It was soaked in chloroform overnight. The contents were transferred to 500ml conical flask, corked well and shaken vigorously using mechanical shaker for one hour. The organic layer (chloroform) was separated and some amount of sodium sulfate was added (to absorb the water). The organic layer was taken in a china dish and evaporated. The solvent was evaporated to maximum extent and the dish was then used for the thin layer chromatographic analysis

Thin Layer Chromatographic analysis

Thin layer chromatography (TLC) method was also used for identification and detection of endosulfan. The concentrated liquid along with control was spotted on the TLC plate using capillary tube. The spotted plate was kept in a TLC chamber containing the solvent system. The eluent used is hexane: chloroform in 1:1 ratio after it reached sufficient height, it was taken from the TLC chamber and was dried in air and diphenylamine was sprayed on the plate and kept in sunlight. A green colour spot appeared on plate.

RESULTS AND DISCUSSION

Thin Layer Chromatography

1/10thof liver and kidney portion (40g) were cut from the 400g human viscera and used for analysis. The developed plates show that the spot corresponding to 1.5 µg of standard endosulfan matches in size with spot corresponding to 1µl of the sample. 15mg of endosulfan was found in sample solution and 150 mg or 0.15g in liver and kidney. In the same way, the total volume of endosulfan was measured. The developed plate shows that the spot corresponding to 2µg of standard endosulfan matches in size with the spot corresponding to 1µl of the sample. 20mg of endosulfan was measured in stomach portion of human viscera. In order to measure the quantity of endosulfan in intestine portion, the developed plate shows that the spot corresponding to 2µg of standard endosulfan matches in size with the spot corresponding to 2µl of sample. In this way, 0.1g of endosulfan was estimated in 450g of intestine portion of human viscera. The result analysis of human viscera by TLC method is listed in Table 1.

Estimation of endosulfan by UV -Visible spectrophotometric method

The UV spectrum of endosulfan is shown in Fig (1).It shows an UV absorption band in the 381 nm region. The five standards of endosulfan were prepared with varying concentrations ranges from (0.5mg/ml to 2.5mg/ml)and the corresponding readings were also noted. The values are given in Table 2.The samples, solutions were prepared and the corresponding readings were noted (Figs. 2 and 3). The values are listed in Table 3.

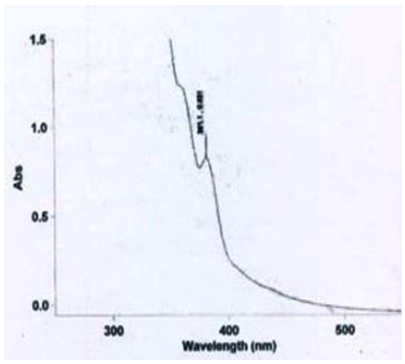

Fig. 1

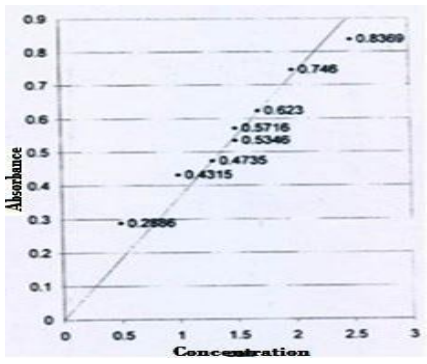

Fig. 2

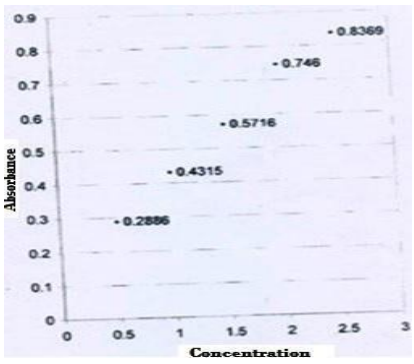

Fig. 3

Table 1: The amount of endosulfan in different portion of human visceraa

Sl. No	Portion of human viscera	Amount of endosulfan (g)	Percentage by weight (%)
1	Liver and Kidney	0.15	0.038
2	Stomach	0.20	0.04
3	Intestine	0.10	0.02

[a]10ml solution was prepared from 1/10[th] of every portion of human viscera

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For calculation, 1/10th of viscera were taken for estimation and the residue (in all the three samples) were made up to 10 ml. The concentration of endosulfan (standard) and endosulfan (sample) were listed in Tables 2 and 3. If 1mlsolution contains 1.7mg of endosulfan, 10ml solution contains 17mg. it is only 1/10th of the solution. So the total volume of endosulfan present in stomach portion was estimated as 170mg. in a similar way, the total amount of endosulfan present in intestine portion and liver and kidney portion was estimated at 130 and 150mg respectively.

Table 2: Concentration of standard endosulfan by UV-Visible spectrophotometer

Sl. No	Standard	Concentration (mg/ml)	Reading
1	1	0.50	0.2886
2	2	1.00	0.4315
3	3	1.50	0.5716
4	4	2.00	0.7460
5	5	2.50	0.8369

Table 3: Concentration of endosulfan in samples from UV-Visible spectrophotometer[a]

S.No	Samples	Concentration mg/ml	Reading	Amount of Endosulfan (mg)
1.	Stomach	1.7	0.6230	170
2.	Intestine	1.3	0.4735	130
3.	Liver & kidney	1.5	0.5346	150

[a]10 ml solution was prepared from 1/10th of every portion of human viscera

In this study, the endosulfan was identified in human viscera by preliminary examinations using spot tests such as nickel-amine reagent and o-tolidine reagent. TLC and UV spectrophotometric methods were used to estimate the quantity (concentration)of poison in the human viscera. From the TLC method 400g of liver and kidney contains 0.15g of endosulfan, 500g of stomach contains 0.2g of endosulfan and 450g of intestine contains 0.1g of endosulfan. From the UV spectrophotometer study, the quantity of endosulfan present in the stomach, intestine and liver and kidney portion was measured as 0.17, 0.13and 0.15g respectively. Both studies show almost same quantity of endosulfan in the human viscera (Table 4). It was also known that the measured quantity is sufficient to cause death of the individual. From the Table 4, it is seen that the TLC and UV spectrophotometric methods gave similar results.

Table 4: Amount of endosulfan in human viscera by TLC and UV-Visible spectrophotometer

Samples	Amount of Endosulfan (mg)	
	TLC	UV
Stomach	200	170
Intestine	100	130
Liver and Kidney	150	150

CONCLUSIONS

From the forensic analysis, the presence of endosulfan was identified in the human viscera using spot tests with nickel amine, o-tolidine reagents and TLC. The spot appeared in the TLC plate is green in colour. By using thin layer chromatography, it was found that the percentage by weight present in the different parts of human viscera such as stomach, liver and kidney and intestine are respectively 0.04, 0.038 and 0.02. The percentage of 0.038% accumulation in liver and kidney is found to be fatal in case of suicidal death. It was further confirmed by UV visible spectrophotometric method. There is similarity among the results obtained by both TLC and UV-Visible spectrophotometry.

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