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Derivative UV Spectrophotometric Detection of Some Common Pesticides in Simulated Samples of Beverages

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Abstract

Pesticides are widely used in India because of their applications in agriculture and household purposes. Due to their high toxicity, over use and easy availability they are encountered in suicidal, accidental and homicidal cases. Poisoning with pesticides are common in developing countries as compared to western countries. Because of large number of cases of deaths due to pesticide poisoning it is imperative to develop sensitive, reliable and accurate methods for their detection from the viscera samples, biological materials and common substrates such as beverages etc. recovered from the crime scene. Derivative UV-Vis spectrophotometry is one of the most widely used techniques for detection of pesticides in viscera and other matrices such as beverages etc. In the present study, pesticides were spiked in beverages like Coffee, Tea, Fruity, Mountain dew and Alcohol and then analysed using derivative UV spectrophotometry. Further, the effect of matrices was studied on the UV spectra of pesticides.

Keywords: Pesticides; UV spectrophotometry; Toxicity; Beverages; Coffee; Tea; Fruity; Mountain dew and Alcohol.

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Introduction

Poisoning is a worldwide problem and pesticide poisoning is one of them and a major problem in developing countries such as India where it is the leading cause of accidental, homicidal and suicidal poisoning. Every year pesticide poisoning gain higher ranking due to its easy availability. Pesticide poisoning is a second major cause of death after road accidents. According to WHO in 2004, about 346,000 people had died due to unintentional poisoning and approximately 37000 had died due to intentional poisoning.[1]

India is an agrarian country where farming is main profession of over 60% population. Most of the farmers are rural based where the literacy rate is low. Pesticides are often used to increase the productivity of the crops which leads to the accidental exposure of farmers to pesticides. Due to unavailability of modern methods of production the financial condition of Indian farmers is poor. Because of easy availability, low cost, and low lethal dose the pesticides are often used for suicides. Another aspect which is related to pesticide poisoning is occupational poisoning; that is poisoning cases restricted to agriculture based professions[2,3]

Pesticides are also used as household insecticides which are sprayed to destroy pests and flies etc. Therefore, poisoning due to insecticides are growing day by day specially among children. Most of the cases of pesticide poisoning are encountered in Northern and North western parts of India because these are the major crops producing states. In these

regions, aluminium phosphide and other pesticides are the main reason of poisoning.[4]

Pesticide poisoning can be suicidal, homicidal or accidental. Almost, one million people had died due to suicidal poisoning in every year.[1] Use of pesticides have been banned since, therefore, there is a sharp decline in pesticide poisoning cases. However, accidental cases of pesticide poisoning are still reported due to uninhibited use of pesticides. Cases of homicidal poisoning are little as compared to suicidal and accidental because of disagreeable odour of pesticides.[5]

Various studies have been conducted for the analysis of different classes of pesticides using UV-VIS spectrophotometry. These include Insecticides [6–15], Fungicides[16–25], Insecticides[6–8,10,12,13,26–30], Herbicides.[21,31] Previous studies show that prevalence of high rate of pesticide poisoning cases in various regions of India such as in Panchkula[1], Jamnagar[32], Bangalore[33], Patiala[34], New Delhi[35], Karnataka[36], Ahmedabad[37], Rohtak[34], Uttar Pradesh [38], Himachal Pradesh[39], Western India[30], Manipur [40], Southern and Central India.[41]

Materials and Methods

Sample collection

Sample of common beverages include Coffee, Tea, Fruity, Soft drink (mountain dew), and Alcohol (Asli santra) (Table 1) and five pesticides like LUVON (Dichlorvous), ROGOR (Dimethoate), BAYGON (Carbamate), FINIT and HILMALA (Malathion) were collected from local market, Patiala. (Table 2 and 3).

Preparation of standards – 3 µl of each pesticide (luvon, rogor, baygon, finit and hilmala) were dissolved in 3 ml of cyclohexane and n- Hexane, respectively.

Preparation of simulate samples

For the preparation of simulated samples two different methods were followed. (Table 4). 20 ml sample of each beverage was taken separately into five beakers and marked accordingly. The beverages were then spiked using 2 ml of each pesticide. After shaking mixture was kept overnight. Next day, 10 ml of sample was taken and extracted 3 times with 15 ml of n- Hexane portions in 60 ml of separating funnel. The hexane layers were then combined and passed through anhydrous sodium sulphate to remove water and evaporated to dryness. The residue so obtained was tested for the presence of pesticides in the samples. Same procedure was followed for all beverages.

Analysis of prepared samples

Prepared samples were analysed using UV 1700 (Shimadzu, Japan). UVProbe 2.0 software was used for recording all readings as well as transformation into first and second derivative. Instrumental parameters used in the present study include a scan range of 225–400 nm in absorbance mode and fast scan speed.

Results

(a) Results of analysis of standards spiked in n-hexane

The zero order spectrum of Luvon in n-hexane shows maxima at 388 nm, 258.5 nm and minima at 253 nm. The zero order spectrum of Rogor in n-hexane shows maxima at 259 nm and 234 nm and minima at 253.5 nm and 230 nm. The zero order spectrum of Baygon in n-hexane shows maxima

Table 1: Name of substrate along with coding

S. No.	Name of substrate	Coding
1	Coffee	C
2	Tea	T
3	Fruity	F
4	Mountain dew	M
5	Alcohol	A

Table 2: Name of Pesticides along with coding

S. No.	Name of pesticides	Coding
1.	Luvon	1
2.	Rogor	2
3.	Baygon	3
4.	Finit	4
5.	Hilmala	5

at 388 nm, 328.5 nm and 258 nm and minima at 354.5 nm, 319.5 nm and 252 nm. The zero order spectrum of Finit in n-hexane shows maxima at 260.5 nm and minima at 251 nm. The zero order spectrum of Hilmala in n-hexane shows maxima at 264.5 nm and minima at 264.5 nm (Fig.1).

The first order spectrum of Luvon in n-hexane shows maxima at 325 nm, 300 nm, 290.5 nm, 256 nm and 230 nm and minima at 335 nm, 307.5 nm, 295 nm, 276 nm and 238.5 nm. The first order spectrum of Rogor in n-hexane shows maxima at 325 nm, 291 nm, 256 nm and 232.5 nm and minima at 336.5 nm, 307.5 nm, 278 nm, 270.5 nm and 241.5 nm. The first order spectrum of Baygon in n-hexane shows maxima at 324 nm and 255.5 nm and minima at 306 nm, 276.5 nm and 235.5 nm. The first order spectrum of Finit in n-hexane shows maxima at 323.5 nm, 302 nm, 290.5 nm and 256 nm and minima at 336 nm, 305.5 nm, 295 nm, 280 nm and 229.5 nm. The first order spectrum of Hilmala in n-hexane shows maxima at 303 nm, 283.5 nm, 271.5 nm and 255 nm and minima at 306 nm, 288.5 nm,

Table 3: Commercial name of pesticides along with composition

S. No.	Commercial Name	Pesticide (Main component)	Composition
1.	Luvon 76% EC (Insecticide)	Dichlorvous	DDVP a.i : 76% w/w Adjuvants : Q.S Total : 100% w/w
2.	Rogor (Insecticide)	Dimethoate	Dimethoate technical (based on 85% w/w a.i) : 35.5% Epichlorohydrin (stabilizer) : 1.0% Emulsifier (nonionic polyoxy ethylene ether) : 8.0% Aromax : 10.5% Xylene : 30.0% Cyclohexanone : 15.0% Total : 100%
3.	Baygon (All insect kiler)	Carbamate	Deltamethrin a.i : 0.05% Allethrin a.i : 0.04% Adjuvants : Q.S% Total : 100%
4.	Finit (Multi insect killer)	Malathion	Pyrothrins : 0.05 w/w Malathion : 1.0 w/w Kerosene base and perfume : Q.S Total : 100% w/w
5.	Hilmala 50 EC (Insecticide)	Malathion	Malathion technical : 52.8% w/w (based on 95% w/w a.i) Stabilizer (Epichlorhydrin) : 1.05 w/w Emulsifier (alkyl aryl Sulphonate and polyoxy ethylene ether) : 5.0% w/w Aromax : 41.2% Total : 100.0% w/w

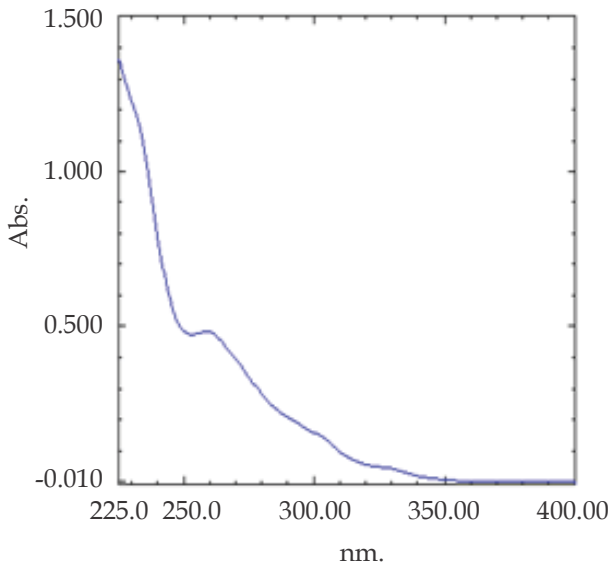

Fig. 1: Zero order spectrum of Luvon in n-hexane

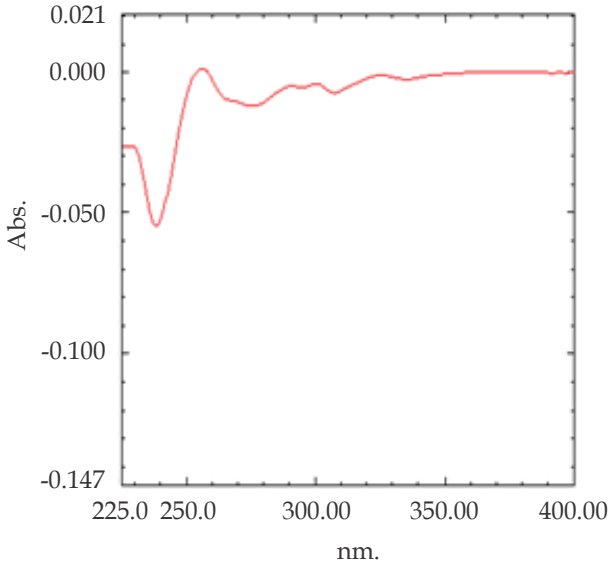

Fig. 2: First order spectrum of Luvon in n-hexane

278.5 nm, 268.5 nm and 229.5 nm (Fig. 2).

The second order spectrum of Luvon in n-hexane shows maxima at 298 nm and 244.5 nm and minima at 303.5 nm, 260.5 nm and 234 nm. The second order spectrum of Rogor in n-hexane shows maxima at 272.5 nm and 249 nm and minima at 260.5 nm and 235.5 nm. The second order spectrum of Baygon in n-hexane shows maxima at 244.5 nm and minima at 232.5 nm. The second order spectrum of Finit in n-hexane shows maxima at 298 nm, 283 nm, 245 nm and 232 nm and minima at 292.5 nm, 260.5 nm and 240.5 nm. The second order spectrum of Hilmala in n-hexane shows maxima at 297 nm, 290.5 nm, 281.5 nm, 269.5 nm, 260 nm, 250 nm and 232 nm and minima at 305 nm, 294 nm, 286.5 nm, 276 nm, 265 nm, 256.5 nm and 240.5 nm (Fig. 3).

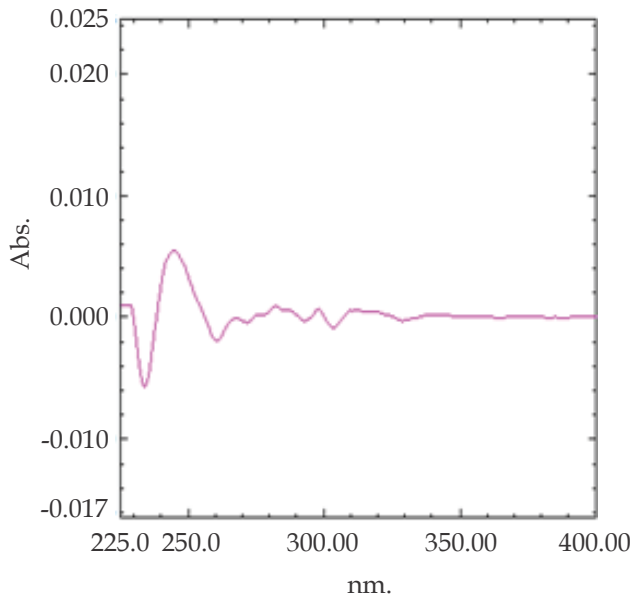

Fig. 3: Second order spectrum of Luvon in n-hexane

Table 4: Name of spiked samples

S. No.	Name of spiked samples	Coding
1	Coffee with Luvon	C 1
2	Coffee with Rogor	C 2
3	Coffee with Baygon	C 3
4	Coffee with Finit	C 4
5	Coffee with Hilmala	C 5
6	Tea with Luvon	T 1
7	Tea with Rogor	T 2
8	Tea with Baygon	T 3
9	Tea with Finit	T 4
10	Tea with Hilmala	T 5
11	Fruity with Luvon	F 1
12	Fruity with Rogor	F 2
13	Fruity with Baygon	F 3
14	Fruity with Finit	F 4
15	Fruity with Hilmala	F 5
16	Mountain dew with Luvon	M 1
17	Mountain dew with Rogor	M 2
18	Mountain dew with Baygon	M 3
19	Mountain dew with Finit	M 4
20	Mountain dew with Hilmala	M 5
21	Alcohol with Luvon	A 1
22	Alcohol with Rogor	A 2
23	Alcohol with Baygon	A 3
24	Alcohol with Finit	A 4
25	Alcohol with Hilmala	A 5

b) Results of analysis of pesticides in various substrates extracted using n- hexane as solvent (Table 5 to 9).

The zero order spectrum of Luvon in coffee (C1) shows maxima at 388 nm and 265.5 nm and minima at 317.5 nm and 256.5 nm. The zero order spectrum of Rogor in coffee (C2) shows maxima at 388 nm and 266 nm and minima at 390 nm, 346 nm, 320.5 nm and 249 nm. The zero order spectrum of Baygon in

Table 5: Results of Luvon extracted using n-hexane from different beverages

Spectrum order	Luvon (neat)		C1		T1		F1		M.D1		A1	
	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)
Zero	388	253	388	317.5	388	392	388	372.5	577	579.5	259	254
	258.5		265.5	256.5	329	354.5	259.5	253	565	567		
						320	232	229	557.5	562		
									520.5	550		
									512	514.5		
									493	508		
									466.5	491		
									458.5	464.5		
									418	456.5		
									394.5	416		
									293	392.5		
									283.5	291		
									262.5	280		
									251.5	260		
									237.5	243		
										229		
First	325	335	299.5	278	300	278.5	325	337	394	398.5	327.5	338
	300	307.5	261.5		259	229.5	291	308	373	377.5	302	309
	290.5	295					256.5	273	363.5	367.5	291	295.5
	256	276					230.5	240	345.5	351.5	256.5	278
	230	238.5							327.5	338.5	233.5	238.5
									303.5	310		
									290	296.5		
									281.5	286.5		
									269	276.5		
									259	266		
									246	252.5		
									232	239		
Second	298	303.5	280.5	276	Nil	Nil	247	260.5	417	413	298.5	304
	244.5	260.5	249	234.5				235	404	396	272.5	293
		234	229.5						391	388.5	245	276
									379	376	229.5	260
									371.5	366		235.5
									360.5	349.5		
									341	338		
									313	307		
									296.5	292		
									289	284		
									276.5	270.5		
									266	263.5		
									259.5	257		
									255	251.5		
									248	234.5		
									230.5			

coffee (C3) shows maxima at 338 nm and 267 nm and minima at 340 nm, 309 nm and 260.5 nm. The zero order spectrum of Finit in coffee (C4) shows maxima at 322 nm and 267.5 nm and 332 nm, 317 nm and 246 nm. The zero order spectrum of Hilmala (C5) shows maxima at 319.5 nm, 311.5 nm, 274 nm and 266 nm and minima at 322 nm, 317 nm, 309.5 nm, 269.5 nm and 246.5 nm (Fig. 4).

Table 6: Results of Rogor extracted using n-hexane from different beverages

Spectrum Order	Rogor (neat)		C2		T2		F2		M.D2		A2	
	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)
Zero	259	253.5	388	390	388	390	388	390	587	584	266	310
	234	230	266	346	266	317	266.5	306.5	577.5	567		240
				320.5		255.5		289.5	558	549.5		
				249				237	546	542		
									533.5	527		
									512	491.5		
									504	482		
									487	474.5		
									476.5	452		
									454	443		
									445.5	425.5		
									433.5	403.5		
									408	384		
									390	358		
									365.5	340.5		
									342.5	254		
									266			
First	325	336.5	324	278	322	278	293.5	278	414.5	410.5	294	278
	291	307.5	302	270	272	269.5	272	270	405	399.5	272	269.5
	256	278	272		263	246	262.5		387.5	369	262.5	
	232.5	270.5	257.5		244		255.5		361.5	355	255.5	
		241.5							349.5	346.5		
									339.5	332.5		
									302	278		
									272	270		
									262.5			
Second	272.5	260.5	280.5	275.5	280.5	287	280.5	275.5	412	415.5	280.5	275.5
	249	235.5	271	266.5	271	275.5	271	266.5	404	407.5	271	266.5
			262	259.5	262	266.5	262		394	396	262	245.5
			250	245	231	259.5	229.5		386	390.5	229.5	
			230.5			245			379	383.5		
									369	376.5		
									357.5	365.5		
									352	354		
									346.5	350		
									339	342.5		
									280.5	333.5		
									271	287.5		
									262	275.5		
									231	266.5		
										259.5		

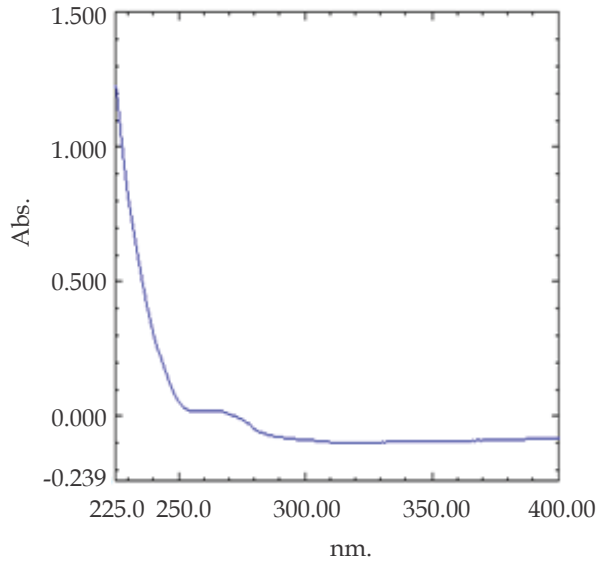

Fig. 4: Zero order spectrum of C1 in n-hexane

Table 7: Results of Baygon extracted using n-hexane from different beverages

Spectrum Order	Baygon (neat)		C3		T3		F3		M.D3		A3	
Zero	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)
	388	354.5	338	340	388	390.5	388	253	598	588.5	265	254.5
	328.5	319.5	267	309	267	308.5	259.5		581	562.5		
	258	252		260.5		261.5	232		564.5	541.5		
									557	528		
									544	488.5		
									540	451		
									512	426		
									472	419		
									428	410		
									421	402.5		
									412	360		
									408	257		
									375			
									259.5			
First	324	306	264	286.5	264.5	287	325.5	338	405.5	400.5	321.5	287.5
	255.5	276.5		236.5		238	302	308.5	396	392.5	302	281.5
		235.5					291	281.5	385.5	378	258.5	233.5
							278	271.5	371.5	369		
							256	240	362.5	356.5		
									354	346.5		
									340	335		
									259	229.5		
Second	244.5	232.5	250.5	266.5	292	267	286	279.5	403.5	406.5	282.5	286.5
			241	244	251	244	275	261	393.5	398.5	270	277.5
				233.5	241	233.5	246	235	381.5	390.5	242.5	266
					230.5				370.5	376		
									361	364		
									339.5	342.5		

The zero order spectrum of Luvon in tea (T1) shows maxima at 388 nm and 329 nm and minima at 392 nm, 354.5 nm and 320 nm. The zero order spectrum of Rogor in tea (T2) shows maxima at 388 nm and 266 nm and minima at 390 nm, 317 nm and 255.5 nm. The zero order spectrum of Baygon in tea (T3) shows maxima at 388 nm and 267 nm and minima at 390.5 nm, 308.5 nm and 261.5 nm. The zero order spectrum of Finit in tea (T4) shows maxima at 388 nm, 322.5 nm and 267.5 nm and minima at 390 nm, 335 nm, 317.5 nm and 247 nm. The zero order spectrum of Hilmala in tea (T5) shows maxima at 388 nm, 319.5 nm, 311.5 nm, 272.5 nm and 265.5 nm and minima at 390 nm, 322.5 nm, 317 nm, 309.5 nm 269.5 nm and 250 nm.

The zero order spectrum of Luvon in fruity (F1) shows maxima at 388 nm, 259.5 nm, 232 nm, 325 nm, 291 nm, 256.5 nm and 230.5 nm and minima at 372.5 nm, 253 nm, 229 nm, 337 nm, 308 nm, 273 nm and 240 nm. The zero order spectrum of Rogor in fruity (F2) shows maxima at 388 nm and 266.5 nm and minima at 390 nm, 306.5 nm, 289.5 nm and 237 nm. The zero order spectrum of Baygon in fruity (F3) shows maxima at 388 nm and 259.5 nm and minima at 253 nm. The zero order spectrum of Finit in fruity (F4) shows maxima at 388 nm and 322 nm and minima at 335 nm and 317.5 nm. The zero order spectrum of Hilmala in fruity (F5) shows maxima at 388 nm, 319 nm, 311.5 nm, 274 nm and 265.5 nm and minima at 389 nm, 317.5 nm, 309.5 nm, 269.5 nm and 248 nm.

Table 8: Results of Finit extracted using n-hexane from different beverages

Spectrum Order	Finit (neat)		C4		T4		F4		M.D4		A4	
	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)
Zero	260.5	251	322	332	388	390	388	335	575.5	591.5	388	312.5
			267.5	317	322.5	335	322	317.5	532.5	549	272	270
				246	267.5	317.5	267.5	245	520	522	267.5	244
						247			510.5	515.5		
									493	502		
									472.5	481.5		
									468	470.5		
									449	463.5		
									441	447		
									398.5	438		
									265	393		
										249		
First	323.5	336	320	326	320	326	320	326	406	410.5	320	295
	302	305.5	288	294.5	288.5	294.5	288.5	294.5	395.5	402	288.5	278.5
	290.5	295	258	279.5	258	278	257.5	279.5	382.5	385.5	256	230
	256	280		234		233		233.5	373	378		
		229.5							363.5	369		
									343	348.5		
									321	336		
									289.5	295		
									257	280		
										234		
Second	298	292.5	297.5	322.5	297.5	292.5	297.5	322.5	411.5	408	282	273.5
	283	260.5	282	292.5	282	273.5	282	292.5	404	399.5	269.5	266
	245	240.5	269.5	273.5	269.5	266.5	269.5	273.5	394.5	389.5	233.5	
	232		237	266.5	236	230.5	236.5	253	386.5	383		
				231.5				231	379	376		
									371	366		
									361	346		
									298	293		
									282.5	273		
									269.5	266		
									236	230.5		

The zero order spectrum of Luvon in mountain dew (M1) shows maxima at 577 nm, 565 nm, 557.5 nm, 520.5 nm, 512 nm, 493 nm, 466.5 nm, 458.5 nm, 418 nm, 394.5 nm, 293 nm, 283.5 nm, 262.5 nm, 251.5 nm and 237.5 nm and minima at 579.5 nm, 567 nm, 562 nm, 550 nm, 514.5 nm, 508 nm, 491 nm, 464.5 nm, 456.5 nm, 416 nm, 392.5 nm, 291 nm, 280 nm, 260 nm, 243 nm and 229 nm. The zero order spectrum of Rogor in mountain dew (M2) shows maxima at 587 nm, 577.5 nm, 558 nm, 546 nm, 533.5 nm, 512 nm, 504 nm, 487 nm, 476.5 nm, 454 nm, 445.5 nm, 433.5 nm, 408 nm, 390 nm, 365.5 nm, 342.5 nm and 266 nm and minima at 584 nm, 567 nm, 549.5 nm, 542 nm, 527 nm, 491.5 nm, 482 nm, 474.5 nm, 452 nm, 443 nm, 425.5 nm, 403.5 nm, 384 nm, 358 nm, 340.5 nm and 254 nm. The zero order spectrum of Baygon in mountain dew (M3) shows

Table 9: Results of Hilmala extracted using n-hexane from different beverages

Spectrum Order	**Hilmala (neat)**		**C5**		**T5**		**F5**		**M.D5**		**A5**	
	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimum (nm)	Maximum (nm)	Minimusm (nm)	Maximum (nm)	Minimum (nm)
Zero	264.5	250.5	319.5	322	388	390	388	389	597	570	388	390
			311.5	317	319.5	322.5	319	317.5	561	550.5	319.5	321.5
			274	309.5	311.5	317	311.5	309.5	548	543.5	311.5	315.5
			266	269.5	272.5	309.5	274	269.5	524	504	274.5	309
				246.5	265.5	269.5	265.5	248	486	479	265.5	269
						250			464	451		247.5
									441.5	436.5		
									408	403		
									379	369.5		
									365	360		
									348.5	346		
									272	270		
									265	251		
First	303	306	318.5	314	303	289	319	314	423.5	428.5	283.5	289
	283.5	288.5	283.5	289	283.5	278.5	303.5	289	413.5	419.5	271.5	278.5
	271.5	278.5	271.5	278.5	271.5	268	283.5	278.5	407	411	262	268
	255	268.5	262	268	261.5	229.5	271.5	268	387.5	399.5	254.5	
		229.5	254	256.5	254.5		261.5	256.5	373	381		
				230.5			254	230.5	361	366.5		
									350	352		
									341	347.5		
									320	332		
									302.5	306		
									283.5	289		
									271	278.5		
									261.5	268		
									254.5	257		
										234		
Second	297	305	315.5	320	290.5	305	315.5	320	421	426417	291	286.5
	290.5	294	308.5	312.5	280.5	294	308.5	312.5	414	409.5	280.5	275.5
	281.5	286.5	291	305	269.5	286	296.5	305.5	404	399.5	269.5	265
	269.5	276	280.5	294	260	275.5	291	294	382.5	377.5	260	256
	260	265	269.5	286	250	265.5	280.5	286.5	368	365.5	251	247
	250	256.5	260	275.5	232	256	269.5	275.5	360	343	231.5	
	232	240.5	250.5	265.5			260	265	339.5	333.5		
			233	255.5			251	256	315.5	320		
				247			233	247	308.5	312.5		
									297	305		
									291	294		
									280.5	286		
									269.5	275		
									260	265		
									242.5	256		
										230.5		

maxima at 598 nm, 581 nm, 564.5 nm, 557 nm, 544 nm, 540 nm, 512 nm, 472 nm, 428 nm, 421 nm, 412 nm, 408 nm, 375 nm and 259.5 nm and minima at 588.5 nm, 562.5 nm, 541.5 nm, 528 nm, 488.5 nm, 451 nm, 426 nm, 419 nm, 410 nm, 402.5 nm, 360 nm and 257 nm. The zero order spectrum of Finit in mountain dew (M4) shows maxima at 575.5 nm, 532.5 nm, 520 nm, 510.5 nm, 493 nm, 472.5 nm, 468 nm, 449 nm, 441 nm, 398.5 nm and 265 nm and minima at 591.5 nm, 549 nm, 522 nm, 515.5 nm, 502 nm, 481.5 nm, 470.5 nm, 463.5 nm, 447 nm, 438 nm, 393 nm and 249 nm. The zero order spectrum of Hilmala in mountain dew (M5) shows maxima at 597 nm, 561 nm, 548 nm, 524 nm, 486 nm, 464 nm, 441.5 nm, 408 nm, 379 nm, 365 nm, 348.5 nm, 272 nm and 265 nm and minima at 570 nm, 550.5 nm, 543.5 nm, 504 nm, 479 nm, 451 nm, 436.5 nm, 403 nm, 369.5 nm, 360 nm, 346 nm, 270 nm and 251 nm.

The zero order spectrum of Luvon in alcohol (A1) shows maxima at 259 nm and minima at 254 nm. The zero order spectrum of Rogor in alcohol (A2) shows maxima at 266 nm and minima at 310 nm and 240 nm. The zero order spectrum of Baygon in alcohol (A3) shows maxima at 265 nm and 254.5 nm. The zero order spectrum of Finit in alcohol (A4) shows maxima at 388 nm, 272 nm and 267.5 nm and minima at 312.5 nm, 270 nm and 244 nm. The zero order spectrum of Hilmala in alcohol (A5) shows maxima at 388 nm, 319.5, 311.5 nm, 274.5 nm and 265.5 nm and minima at 390 nm, 321.5 nm, 315.5 nm, 309 nm 269 nm and 247.5 nm.

The first order spectrum of Luvon in coffee (C1) shows maxima at 299.5 nm and 261.5 nm and minima at 278 nm. The first order spectrum of Rogor in coffee (C2) shows maxima at 324 nm, 302 nm, 272 nm and 257.5 nm and minima at 278 nm and 270 nm. The first order spectrum of Baygon in coffee (C3) shows maxima at 264 nm and minima at 286.5 nm and 236.5 nm. The first order spectrum of Finit in coffee (C4) shows maxima at 320 nm, 288 nm and 258 nm and minima at 326 nm, 294.5 nm, 279.5 nm and 234 nm. The first order spectrum of Hilmala in coffee (C5) shows maxima at 318.5 nm, 283.5 nm, 271.5 nm, 262 nm and 254 nm and minima at 314 nm, 289 nm, 278.5 nm, 268 nm, 256.5 nm and 230.5 nm (Fig. 5).

The first order spectrum of Luvon in tea (T1) shows maxima at 388 nm and 329 nm and minima at 278.5 nm and 229.5 nm. The first order spectrum of Rogor in tea (T2) shows maxima at 322 nm, 272 nm, 263 nm and 244 nm and minima at 278 nm, 269.5 nm and 246 nm. The first order spectrum of Baygon in tea (T3) shows maxima at 264.5 nm and minima at 287 nm and 238 nm. The first order spectrum of Finit in tea (T4) shows maxima at 320 nm, 288.5 nm and 258 nm and minima at 326 nm, 288.5 nm and 258 nm. The first order spectrum of Hilmala in tea (T5) shows maxima at 303 nm, 283.5 nm, 271.5 nm, 261.5 nm and 254.5 nm and minima at 289 nm, 278.5 nm, 268 nm and 229.5 nm.

The first order spectrum of Luvon in fruity (F1) shows maxima at 325 nm, 291 nm, 256.5 nm and 230.5 nm and minima at 337 nm, 308 nm, 273 nm and 240 nm. The first order spectrum of Rogor in fruity (F2) shows maxima at 293.5 nm, 272 nm, 262.5 nm and 255.5 nm and minima at 278 nm and 270 nm. The first order spectrum of Baygon in fruity (F3) shows maxima at 325.5 nm, 302 nm, 291 nm, 278 nm and 256 nm and minima at 338 nm,

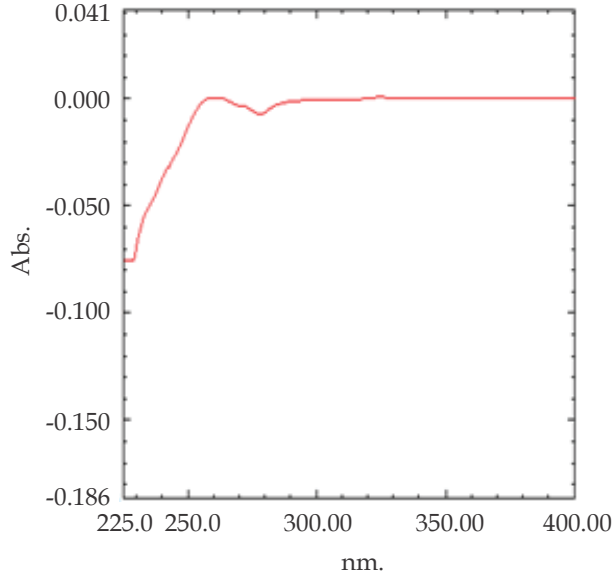

Fig. 5: First order spectrum of C1 in n-hexane

308 nm, 281.5 nm, 271.5 nm and 240 nm. The first order spectrum of Finit in fruity (F4) shows maxima at 320 nm, 288.5 nm and 257.5 nm and minima at 326 nm, 294.5 nm, 279.5 nm and 233.5 nm. The first order spectrum of Hilmala in fruity (F5) shows maxima at 319 nm, 303.5 nm, 283.5 nm, 271.5 nm, 261.5 nm and 254 nm and minima at 314 nm, 289 nm, 278.5 nm, 268 nm, 256.5 nm and 230.5 nm.

The first order spectrum of Luvon in mountain dew (M1) shows maxima at 394 nm, 374 nm, 363.5 nm, 345.5 nm, 327.5 nm, 303.5 nm, 290 nm, 281.5 nm, 269 nm, 259 nm, 246 nm and 232 nm and minima at 298.5 nm, 277.5 nm, 367.5 nm, 351.5 nm, 338.5 nm, 310 nm, 296.5 nm, 286.5 nm, 276.5 nm, 266 nm, 252.5 nm and 239 nm. The first order spectrum of Rogor in mountain dew (M2) shows maxima at 414.5 nm, 404 nm, 387.5 nm, 361.5 nm, 349.5 nm, 339.5 nm, 302 nm, 272 nm and 262.5 nm and minima at 410.5 nm, 399.5 nm, 369 nm, 355 nm, 346.5 nm, 332.5 nm, 278 nm and 270 nm. The first order spectrum of Baygon in mountain dew (M3) shows maxima at 405.5 nm, 396 nm, 385.5 nm, 371.5 nm, 362.5 nm, 354 nm, 340 nm and 259 nm and minima at 400.5 nm, 392.5 nm, 378 nm, 369 nm, 356.5 nm, 346.5 nm, 335 nm and 229.5 nm. The first order spectrum of Finit in mountain dew (M4) shows maxima at 406 nm, 395.5 nm, 382.5 nm, 373 nm, 363.5 nm, 343 nm, 321 nm, 289.5 nm and 257 nm and minima at 410.5 nm, 402 nm, 385.5 nm, 378 nm, 369 nm, 348.5 nm, 336 nm, 295 nm, 280 nm and 234 nm. The first order spectrum of Hilmala in mountain dew (M5) shows maxima at 423.5 nm, 413.5 nm, 407 nm, 387.5 nm, 373 nm, 361 nm, 350 nm, 341 nm, 320 nm, 302.5 nm, 283.5 nm, 271 nm, 261.5 nm and 254.5 nm and minima at 428.5 nm, 419.5 nm, 411 nm, 399.5 nm, 381 nm, 366.5 nm, 352 nm, 347.5 nm, 332 nm, 306 nm, 289 nm, 278.5 nm, 268 nm, 257 nm and 234 nm.

The first order spectrum of Luvon in alcohol (A1) shows maxima at 327.5 nm, 302 nm, 291 nm, 256.5 nm and 233.5 nm and minima at 338 nm, 309 nm, 295.5 nm, 278 nm and 238.5 nm. The first order spectrum of Rogor in alcohol (A2) shows maxima at 294 nm, 272 nm, 262.5 nm and 255.5 nm and minima at 278 nm and 269.5 nm. The first order spectrum of Baygon in alcohol (A3) shows maxima at 321.5 nm, 302 nm and 258.5 nm and minima at 287.5 nm, 281.5 nm and 233.5 nm. The first order spectrum of Finit in alcohol (A4) shows maxima at 320 nm, 288.5 nm and 256 nm and 295 nm, 278.5 nm and 230 nm. The first order spectrum of Hilmala in alcohol (A5) shows maxima at 283.5 nm, 271.5 nm, 262 nm and 254.5 nm and minima at 289 nm, 278.5 nm and 268 nm.

The second order spectrum of Luvon in coffee (C1) shows maxima at 280.5 nm, 249 nm and 229.5 nm and minima at 276 nm and 234.5 nm. The second order spectrum of Rogor in coffee (C2) shows maxima at 280.5 nm, 271 nm, 262 nm, 250 nm and 230.5 nm and minima at 275.5 nm, 266.5 nm, 259.5 nm and 245 nm. The second order spectrum of Baygon in coffee (C3) shows maxima at 250.5 nm and 241 nm and minima at 266.5 nm, 244 nm and 233.5 nm. The second order spectrum of Finit in coffee (C4) shows maxima at 297.5 nm, 282 nm, 269.5 nm and 237 nm and minima at 322.5 nm, 292.5 nm, 273.5 nm, 266.5 nm and 231 nm. The second order spectrum of Hilmala in coffee (C5) shows maxima at 315.5 nm, 308.5 nm, 291 nm, 280.5 nm, 269.5 nm, 260 nm, 250.5 nm and 233 nm and minima at 320 nm, 312.5 nm, 305 nm, 294 nm, 286 nm, 275.5 nm, 265.5 nm, 255.5 nm and 247 nm. (Fig. 6).

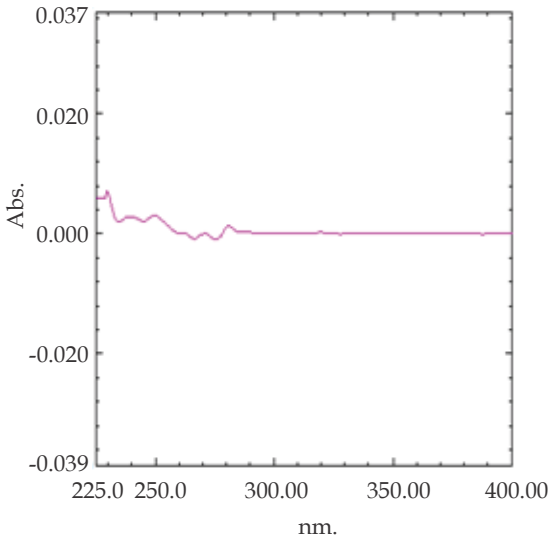

Fig. 6: Second order spectrum of C1 in n-hexane

The second order spectrum of Luvon in tea (T1) is nil. The second order spectrum of Rogor in tea (T2) shows maxima at 280.5 nm, 271 nm, 262 nm and 231 nm and minima at 287 nm, 275.5 nm, 266 nm, 259.5 nm and 245 nm. The second order spectrum of Baygon in tea (T3) shows maxima at 292 nm, 251 nm, 241 nm and 230.5 nm and minima at 267 nm, 244 nm and 233.5 nm. The second order spectrum of Finit in tea (T4) shows maxima at 297.5 nm, 282 nm, 269.5 nm and 236 nm and minima at 292.5 nm, 273.5 nm, 266.5 nm and 230.5 nm. The second order spectrum of Hilmala in tea (T5) shows maxima at 290.5 nm, 280.5 nm, 269.5 nm, 260 nm, 250 nm and 232 nm and minima at 305 nm, 294 nm, 286 nm, 275.5 nm, 265.5 nm and 256 nm.

The second order spectrum of Luvon in fruity (F1) shows maxima at 247 nm and 260.5 nm and 235 nm. The second order spectrum of Rogor in fruity (F2) shows maxima at 280.5 nm, 271 nm, 262 nm and 229.5 nm and minima at 275.5 nm and 266.5 nm. The second order spectrum of Baygon in fruity (F3) shows maxima at 286 nm, 275 nm and 246 nm and minima at 279.5 nm, 261 nm and 235 nm. The second order spectrum of Finit in fruity (F4) shows maxima at 297.5 nm, 282 nm, 269.5 nm and 236.5 nm and minima at 322.5 nm, 292.5 nm, 273.5 nm, 253 nm and 231 nm. The second order spectrum of Hilmala in fruity (F5) shows maxima at 315.5 nm, 308.5 nm, 296.5 nm, 291, 280.5, 269.5 nm, 260 nm, 251 nm and 233 nm and 320 nm, 312.5 nm, 305.5 nm, 294 nm, 286.5 nm, 275.5 nm, 265 nm, 256 nm and 247 nm.

The second order spectrum of Luvon in mountain dew (M1) shows maxima at 417 nm, 404 nm, 391 nm, 379 nm, 371.5 nm, 360.5 nm, 341 nm, 313 nm, 296.5 nm, 289 nm, 276.5 nm, 266 nm, 259.5 nm, 255 nm, 248 nm and 230.5 nm and minima at 413 nm, 396 nm, 388.5 nm, 376 nm, 366 nm, 349.5 nm, 338 nm, 307 nm, 292 nm, 284 nm, 270.5 nm, 263.5 nm, 257 nm, 251.5 nm and 234.5 nm. The second order spectrum of Rogor in mountain dew (M2) shows maxima at 412 nm, 404 nm, 394 nm, 386 nm, 379 nm, 369 nm, 357.5 nm, 352 nm, 346.5 nm, 339 nm, 280.5 nm, 271 nm, 262 nm and 231 nm and minima at 415.5 nm, 407.5 nm, 396 nm, 390.5 nm, 383.5 nm, 376.5 nm, 365.5 nm, 354 nm, 350 nm, 342.5 nm, 333.5 nm, 287.5 nm, 275.5 nm, 266.5 nm, 259.5 nm. The second order spectrum of Baygon in mountain dew (M3) shows maxima at 403.5 nm, 393.5 nm, 381.5 nm, 370.5 nm, 361 nm and 339.5 nm and minima at 406.5 nm, 398.5 nm, 390.5 nm, 376 nm, 364 nm, 342.5 nm. The second order spectrum of Finit in mountain dew (M4) shows maxima at 411.5 nm, 404 nm, 394.5 nm, 386.5 nm, 379 nm, 371 nm, 361 nm, 298 nm, 282.5 nm, 269.5 nm and 236 nm and minima at 408 nm, 399.5 nm, 389.5 nm, 383 nm, 376 nm, 366 nm, 346 nm, 293 nm, 273 nm, 266 nm and 230.5 nm. The second order spectrum of Hilmala in mountain dew (M5) shows maxima at 421 nm, 414 nm, 404 nm, 382.5 nm, 368 nm, 360 nm, 339.5 nm, 315.5 nm, 308 nm, 297 nm, 291 nm, 280.5 nm, 269.5 nm, 260 nm and 242.5 nm and minima at 426 nm, 417 nm, 409.5 nm, 399.5 nm, 377.5 nm, 365.5 nm, 343 nm, 333.5 nm, 320 nm, 312.5 nm, 305 nm, 294 nm, 286 nm, 275 nm, 265 nm, 256 nm and 230.5 nm.

The second order spectrum of Luvon in alcohol (A1) shows maxima at 298.5 nm, 272.5 nm, 245 nm and 229.5 nm and minima at 304 nm, 293 nm, 276 nm, 260 nm and 235.5 nm. The second order spectrum of Rogor in alcohol (A2) shows maxima at 280.5 nm, 271 nm, 262 nm and 229.5 nm and minima at 275.5 nm, 266.5 nm and 245.5 nm. The second order spectrum of Baygon in alcohol (A3) shows maxima at 282.5 nm, 270 nm and 242.5 nm and minima at 286.5, 277.5 nm and 266 nm. The second order spectrum of Finit in alcohol (A4) shows maxima at 282 nm, 269.5 nm and 233.5 nm and minima at 273.5 nm and 266 nm. The second order spectrum of Hilmala in alcohol (A5) shows maxima at 291 nm, 280.5 nm, 269.5 nm, 260 nm, 251 nm and 231 nm and minima at 286.5 nm, 275.5 nm, 265 nm, 256 and 247 nm.

Conclusion

Derivative UV spectroscopy is an efficient technique for the identification of the pesticides when encountered in common beverages. The zero order can be used for elimination purpose, whereas, the first and second order derivatives can be used for more accurate identification. Shifts in peaks can be observed due to some interference of the matrices, however, the identification is possible even in cases where the pesticides are found in beverages. Derivative Ultraviolet spectrophotometry is less time consuming, easier to use, non-destructive, and sensitive technique with good accuracy and precision. The cost of analysis per samples is less compared to other techniques such as GC-MS, etc. Therefore, it can be successfully used for elimination as well as identification process.

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Conflict of interest: None to declare

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Original Article

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Fourier Transform Infrared Spectroscopic Characterisation of Some Common Antidepressans in Pharmaceutical Preparations

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Abstract

The objective of this study was to analyse some common antidepressants in pharmaceutical preparations. The antidepressants taken for analysis were Selective Serotonin Reuptake Inhibitors [SSRIs]. SSRIs were associated with the significantly lower risk of toxicity, but large number of deaths from SSRIs has occurred in combination with other drugs specifically tricyclic and tetracyclic antidepressants. There has been a lot of work in the characterisation of antidepressants in pharmaceutical preparations but still a rapid and reliable positive qualitative identification of SSRIs needs to be developed. In the present investigation, Fourier Transform Infra Red [FTIR] spectrophotometer in transmission and Attenuated Total Reflectance [ATR] mode was used to qualitatively identify pharmaceutical preparation consisting of Proxetine, Sertraline, Escitalopram, Fluvoxamine. The results suggested that both the modes provided greater sensitivity but the ATR mode has great potential for the characterisation of SSRIs.

Keywords: Pharmaceutical; Selective Serotonin Reuptake Inhibitors [SSRIs]; Attenuated Total Reflectance [ATR].; Fourier Transform Infra Red [FTIR]; Antidepressants.

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Introduction

The SSRIs block neuronal transport of 5-HT (Serotonin) both immediately and chronically, leading to complex secondary responses. Increased synaptic availability of 5-HT results in stimulation of a large number of postsynaptic 5-HT receptor types, which may contribute to adverse effects characteristic of this class of drugs, including GI effects (nausea and vomiting) and sexual effects (delayed or impaired orgasm). Stimulation of 5-HT_{2C} receptors may contribute to the agitation or restlessness sometimes induced by SSRIs.[1]

Several techniques like ultraviolet/visible spectrophotometry, fluorimetry, electroanalytical techniques, chromatographic methods (thin- layer chromatography, gas chromatography and high-performance liquid chromatography), capillary electrophoresis and vibrational spectroscopies are the main techniques that have been used for the quantitative and qualitative analysis of pharmaceutical compounds (like antidepressants). Although simple techniques such as UV/VIS spectrophotometry and TLC are still extensively employed, HPLC is the most popular instrumental technique used for the analysis of pharmaceuticals. In the area of pharmaceutical analysis showed a trend in the application of techniques increasingly rapid such as ultra performance liquid chromatography and the use of sensitive and specific detectors as mass spectrometers.[2]

Materials and Methods

Collection of Samples

All the samples of drugs were purchased from the medical store in the form of pharmaceutical preparations. The description of the samples analyzed is given in Table 1.

Sample Preparation

About 2 mg of the finely powdered dry material was mixed with 200 mg of KBr, the same was grounded manually in an agate mortar and pressed into a thin disc the idea was to produce a disc as nearly transparent as possible. Small amount of the powdered sample was placed on the ZnSe atr ptalform of the FTIR spectrometer.

Experimental

FTIR and ATR Spectroscopy: The drugs mentioned in table 1 were analyzed with FTIR spectrophotometer, spectrum 2 Perkin Elmer; over the region of mid IR 4000-400 cm^{-1} at transmission mode using standard KBr pellet method. The ATR spectra was recorded with UATR (with ZnSe crystal) over the region of mid IR 4000-200 cm^{-1}.

Results and Discussion

Qualitative analysis using FTIR and ATR spectroscopy

By observing the position and shape of the vibrational bands in FTIR and ATR spectra of the drugs Proxetine, Sertraline, Escitalopram and Fluvoxamine a satisfactory vibrational band assignment has been made. They are summarized in Tables 2, 3 and 4.

In Table 2 there is description of the FTIR spectra of drugs Proxetine, Sertraline, Escitalopram and Fluvoxamine. In case of Proxetine [sample code A], considering the N-H group of proxetine the vibrational modes of N-H stretching occurs at 3469 cm^{-1}. The aromatic ring [C=C] stretch occur at frequency 1662 cm^{-1} and the C-H stretch and C-H bend of aromatic ring occur at frequency 2915.8 cm^{-1} and 889.23 cm^{-1} respectively. The vibration modes of C-F stretch occurs at 1442.4 cm^{-1}. The ether group [C-O-C] vibrates at frequency 1031cm^{-1}. In case of sertraline [sample code B], the N-H group stretch occurs at frequency 3413 cm^{-1}. The aromatic ring [C=C] stretch occur at frequency 1648.2 cm^{-1} and the C-H stretch and C-H bend of aromatic ring occur at frequency 2921.4 cm^{-1} and 883.67 cm^{-1} respectively. The aliphatic C-H stretch occurs at frequency 1467.5 cm^{-1}. N-C stretch occurs at frequency 1031 cm^{-1}. In case of Escitalopram [sample code C], N:::C stretch occur at 2229.2 cm^{-1} frequency. The aromatic ring [C=C] stretch occur at frequency 1648.2cm^{-1} and the C-H stretch and C-H bend of aromatic ring occur at frequency 2904.2 cm^{-1} and 764.13 cm^{-1} respectively. The C-F bond stretch occurs at frequency 1428.5 cm^{-1}. The ether group [C-O-C] in escitalopram stretch at frequency 1336.8 cm^{-1}. N-C bond stretches at frequency 1022.7 cm^{-1}. In last case of fluvoxamine [sample code D], N-H bond stretches at 3396.8 cm^{-1}, aromatic ring [C=C] stretch occur at frequency 1620.4 cm^{-1}, N-C stretches at frequency 1081 cm^{-1} and the ether group [C-O-C] stretch occur at frequency 1017 cm^{-1}.

In Table 3 there is description of the ATR spectra of drugs Proxetine, Sertraline, Escitalopram and Fluvoxamine. In case of Proxetine [sample code A], considering the N-H group of proxetine the vibrational modes of N-H stretching occurs at 3285.63 cm^{-1}. The aromatic ring [C=C] stretch occur at frequency 1650.03 cm^{-1} and the C-H stretch and C-H bend of aromatic ring occur at frequency 2916.87 cm^{-1} and 892.1 cm^{-1} respectively. The vibration modes of C-F stretch occur at 1418.4 cm^{-1}. The ether group [C-O-C] vibrates at frequency 1032.4 cm^{-1}. In case of Sertraline [sample code B], the N-H group stretch occurs at frequency 3318.3 cm^{-1}. The C-H stretch and C-H bend of aromatic ring occur

Table 1: Description of the drugs analyzes

Sample Code	Generic Name	Category	Composition	Manufacturer
A	Proxetine	Anti- depressant	Anhydrous Proxetine Hydrochloride, 12.5 mg	Zentiva pharmasecutic-al
B	Sertraline	Anti- depressant	Sertraline Hydrochloride, 50 mg	Ranbaxy laboratory limited
C	Escitalopram	Anti- depressant	Escitalopram Oxalate, 10 mg	Akums drugs and pharmaceutical ltd. Haridwar
D	Fluvoxamine	Anti- depressant	Fluvoxamine Malate, 50 mg	Sunpharma Sikkim, Sikkim

at frequency 2856 cm^{-1} and 1018.6 cm^{-1} respectively. N-C stretch occurs at frequency 1137.8 cm^{-1}. In case of Escitalopram [sample code C], the aromatic ring [C=C] stretch occur at frequency 1631.23 cm^{-1} and the C-H stretch of aromatic ring occurs at frequency 2900.44 cm^{-1}. The C-F bond stretch occurs at frequency 1424 cm^{-1}. The ether group [C-O-C] in escitalopram stretch at frequency 1336.63 cm^{1} N-C bond stretches at frequency 1018.01 cm^{-1}. In last case of fluvoxamine [sample code D], N-H bond stretches at 3393.5 cm^{-1}, aromatic ring [C=C] stretch occur at frequency 1619.5 cm^{-1}, N-C stretches at frequency 1075.6 cm^{-1} and the ether group [C-O-C] stretch occur at frequency 1013.4 cm^{-1}.

In Table 4 there is description of comparison of transmission mode and the ATR mode spectra of drugs Proxetine, Sertraline, Escitalopram

Table 2: FTIR spectral readings of drugs Proxetine, Sertraline, Escitalopram, Fluvoxamine.

Sample Code	Generic Name	FTIR Frequency cm-1	Vibrational band assignment
A	Proxetine	3469	N-H stretch
		2915.8	C-H stretch aromatic
		1662	C=C stretch
		1442.4	C-F stretch
		1031	C-O-C stretch
		889.23	C-H bend aromatic
B	Sertraline	3413	N-H stretch
		2921.4	C-H stretch aromatic
		1648.2	C=C stretch aromatic
		1400.7	C-H bend aliphatic
		883.67	N-C stretch
		675.18	C-H bend aromatic
C	Escitalopram	2904.7	C-H stretch aromatic
		2229.2	N:::C stretch
		1648.2	C=C stretch aromatic
		1428.5	C-F stretch
		1336.8	C-O-C stretch
		1022.7	N-C stretch
		764.13	C-H bend aromatic
D	Fluvoxamine	3396.8	N-H stretch
		1620.4	C=C stretch aromatic
		1081	N-C stretch
		1017	C-O-C stretch

Table 3: ATR spectral readings of drugs Proxetine, Sertraline, Escitalopram, Fluvoxamine.

Sample Code	Generic Name	ATR Frequency cm-1	Vibrational band assignment
A	Proxetine	3285.63	N-H stretch
		2916.87	C-H stretch aromatic
		1650.03	C=C stretch
		1418.67	C-F stretch
		1032.40	C-O-C stretch
		892.1	C-H bend aromatic
B	Sertraline	3318.3	N-H stretch
		2856	C-H stretch aromatic
		1469.3	C-H bend aliphatic
		1018.6	N-C stretch
		829.51	C-H bend aromatic
C	Escitalopram	2900.44	C-H stretch
		1631.23	C=C stretch aromatic
		1424	C-F stretch
		1336.63	C-O-C stretch
		1018.01	N-C stretch
D	Fluvoxamine	3393.5	N-H stretch
		1619.5	C=C stretch aromatic
		1075.6	N-C stretch
		1013.4	C-O-C stretch

Table 4: FTIR and ATR spectrum comparison of drugs Proxetine, Sertraline, Escitalopram, Fluvoxamine.

Sample Code	Generic Name	FTIR Frequency cm^{-1}	ATR Frequency cm^{-1}	Vibrational band assignment
A	Proxetine	3469	3285.63	N-H stretch
		2915.8	2916.87	C-H stretch aromatic
		2648.9		
		1662	1650.03	C=C stretch
		1442.4	1418.67	C-F stretch
		1031	1032.40	C-O-C stretch
		889.23	892.1	C-H bend aromatic
B	Sertraline	3413	3318.3	N-H stretch
		2921.4	2856	N^+H_2 stretch
		2685	2679	
		2473.8	2451.4	
		2364.6		
		1648.2		C=C stretch aromatic
		1681.4		C-H bend aliphatic
		1467.5	1469.3	
		1400.7		
		1133.8	1137.8	N-C stretch
		1031	1018.6	C-H bend aromatic
		883.67	829.51	
		675.18		
C	Escitalopram	3385.6	3266.43	C-H stretch
		2904.7	2900.44	N:::C stretch
		2229.2		C=C stretch aromatic
		1648.2	1631.23	C-F stretch
		1428.5	1424	C-O-C stretch
		1336.8	1336.63	N-C stretch
		1022.7	1018.01	C-H bend aromatic
		764.13		
D	Fluvoxamine	3396.8	3393.5	N-H stretch
		2145.6		
		1695.4		
		1620.4	1619.5	C=C stretch aromatic
		1081	1075.6	N-C stretch
		1017	1013.4	C-O-C stretch

and Fluvoxamine, the basic difference is that in transmission mode there was intense region at higher wavenumbers but at lower wavenumbers the intensity was not found to be good. But while working with UATR, the peaks at lower wavenumbers are more intense as compared to the higher wavenumbers.

It is concluded from the present study that FTIR spectroscopy has great potential as an analytical tool for the characterisation of SSRIs in pharmaceutical preparations. Spectrum in Transmission and ATR modes can be very well assigned to different functional groups present in to the samples. Recording of the spectra in to the ATR mode was found to be much more beneficial because of the presence of more intense peaks at lower wavelengths. Another added advantage in this mode was no sample preparation required.

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Original Article

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Separation and Detection of Quizalofop-Ethyl Herbicide by Thin-Layer Chromatography

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Abstract

Herbicides are frequently used in agriculture for control of weeds in crops. Quizalofop-ethyl is a postemergence herbicide widely used for control of grassy weeds in crops, intended for human consumption. The general population may be exposed to quizalofop-ethyl via ingestion of food or drinking water. Sometimes it is misused for suicidal poisoning. A systematic analysis of herbicides acting as poison in human body is carried out by forensic toxicologists. Their method involves screening of poison followed by its instrumental assay. Therefore a simple, rapid, sensitive and reliable thin- layer chromatographic method for detection of quizalofop-ethyl is presented. The quizalofop-ethyl contain quinoxaline ring in its structure having –N=C-CH=N --group. These react with Dragendorff`s reagent to give orange-red coloured spot in yellow background. The detection limit was found to be 10 µg per spot (20 µg/cm^2). The constituents of viscera (amino acids, peptides, proteins, etc.) do not interfere in the test.

Keywords: Herbicides; Quizalofop-ethyl; Thin-Layer Chromatography; Spray Reagent; Dragendorff`s.

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Introduction

Quizalofop-ethyl (2-[4-{6-chloro-2-quinoxalinyl} oxy] propionic acid ethyl ester) is a herbicide widely used to control grassy weeds in broad leaved crops.[1] But the World Health Organisation has classified it as moderately hazardous poison (Class II).[2] Owing to its ready availability; it is sometimes misused for acute intentional self poisoning. Such cases are referred to forensic science laboratories for detection of poison in biological materials.

Several instrumental methods[3-7] are reported in literature for isolation and quantitation of quizalofop-ethyl. Though the instrumental methods are sensitive they require elaborate instrumental assay. Therefore thin-layer chromatography (TLC) is preferred for screening the poisons, due to its simplicity and rapidity. We report Dragendorff`s reagent for detection of quizalofop-ethyl herbicide.

Materials and Methods

All chemicals used were of analytical reagent grade and quizalofop-ethyl was obtained from Dhanuka Pesticides, Gurgaon. Distilled water was used throughout. Standard solution of quizalofop-ethyl 2mg/ml was prepared in benzene.

Spray reagents: (i) Dragendorff`s reagent (a) Mix together 2 gm bismuth sub nitrate, 25 ml acetic acid and make to 100 ml with water. (b) Dissolve

40 gm potassium iodide in 100 ml water. Mix together 10 ml of (a) and 10 ml of (b) and use this as spray reagent.

(ii) Aqueous nitric acid 50% v/v.

Thin-layer Chromatography

Standard glass TLC plates (10 x 15 cm) were coated with slurry of silica gel G (Sisco Reasearch Laboratories, Mumbai) in water (1:2) to produce uniform 0.25 mm layers. These were left to dry at room temperature. Plates were activated by heating in oven at 100°C for ca. 1 hour. Before use the plates were stored in desiccators. Standard solutions of quizalofop-ethyl (1 µL, 5 µL and 10 µL) were spotted 1.5 cm from the bottom of the plate by means of a micropipette and spots were left to dry in air. Plates were then developed in a presaturated TLC chamber (development time 20 min) using two solvent systems, (I) hexane:acetone (8+2) and (II) chloroform:ether (7+3) at 25°C temperature, by ascending technique. After the mobile phase has migrated to ca. 10 cm, the plate was removed from the chamber and left to dry at room temperature. It was then sprayed uniformly with Dragendorff`s reagent followed by dil. nitric acid. Orange- red coloured spots for 5 µL and 10 µL were found in yellow background at R_F 0.55 in solvent system (I) and at R_F 0.92 in solvent system (II). The detection limit for quizalfop-ethyl was 10 µg per spot (20 µg/cm^2).

Recovery of quizalofop-ethyl from biological materials

For the semi-quantitative determination of quizalofop-ethyl, 2 mg of herbicide was added to ca. 50 gm of minced visceral tissue (stomach, intestine, liver, etc.) and kept for a day. The herbicide was then extracted with benzene as its recovery is more in benzene.[8] The solvent was evaporated at room temperature and the residue was dissolved in 1 ml ethanol. A 10 µl volume of this solution was spotted on an activated plate along with 10 µL each of standard solution containing 16.0 µg, 18.0 µg, 20.0 µg and 22.0 µg of quizalofop-ethyl. The plate was then developed as described above. The intensity of the orange- red spot produced by the visceral extract was comparable to that of the spot corresponding to 18 µg of quazalofop-ethyl (average of three experiments). Hence the recovery was ca. 90%.

Results and Discussion

The quizalofop-ethyl has a quinoxaline ring in its structure. It is heterocyclic in nature having two nitrogen at 1 and 4 positions in pyrazine ring. These are the reactive nitrogen which combines with heavy metal atom (Bi I_4) present in Dragendorff`s reagent to form ion pairs. This ion pair forms insoluble orange-red coloured complex. The colour of the spot on TLC remains stable for couple of days. The solvent system gives compact spots. The constituents of viscera (amino acids, peptides, proteins, etc.) generally co extracted with quizalofop-ethyl do not interfere with the test. Hence the proposed reagent, owing to its sensitivity can be useful for detection and semi-quantitative determination of quazalofop-ethyl in biological materials.

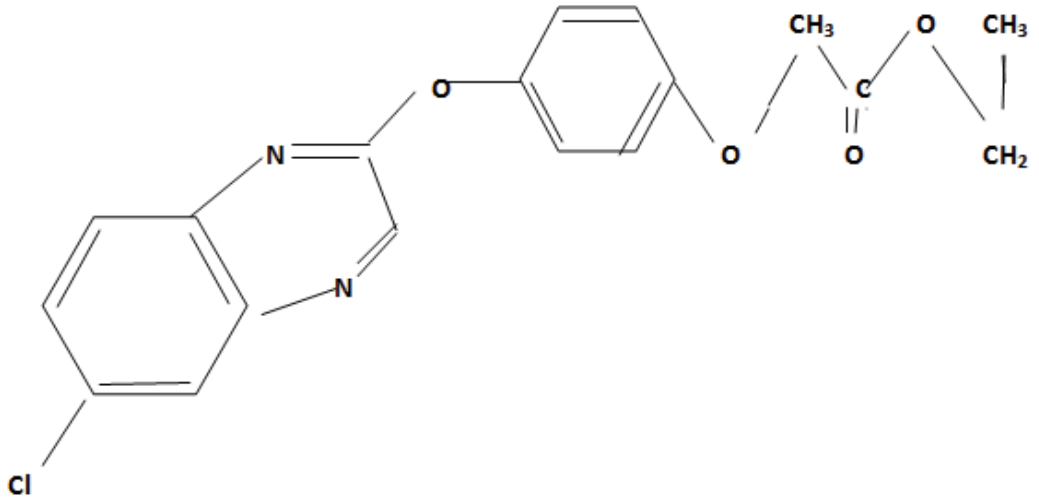

Fig. 1: Chemical structure of Quizalofop-ethyl

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Assessment of Acute and 28-Days Repeated Dose Sub-Acute Toxicity Study of Selected Ultra-Diluted Preparations in Wistar Rats

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Abstract

Objective: The aim of this work was to investigate the acute and sub-acute toxicity studies of the homeopathic drugs, causticum, calcerea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja in experimental models. *Materials and Methods:* Animals were divided into groups (n=5). Homeopathic drugs were administered to rats for 14 and 28 days to assess the toxicological profile. Acute and sub-acute studies were carried out according to OECD 425, 407 guidelines. Behavioural parameters and mortality rate were assessed in acute toxicity study. The sub-acute was performed for a study period of 28 days and afterwards animals were sacrificed to carry out biochemical and haematological estimations. The histopathological analysis of all vital organs was done with haematoxylin and eosin staining to assess the anatomical damage involved. *Results:* Findings of the study revealed that the administration of homeopathic drugs at single bolus dose in acute toxicity study there were no mortality or any signs of toxicity observed after oral administration of drugs up to the dose level that of 2000 µl in rats. So, the LD50 was found to be greater than 2000 ul/100g body weight. Sub-acute toxicity study was conducted for a period of 28 days and we noted that the there were no pathological or biochemical alterations found. *Conclusion:* Study inferred that homeopathic drugs are safe on acute and sub-acute administration for 14 and 28 days respectively. The safety profile is established in experimental animals and further data can be corroborated in humans.

Keywords: Toxicity; Homeopathic drugs; Histopathology; Mortality; Biochemical.

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Introduction

A new system of therapeutics is based on the idea that substances simulating the symptoms of illness could aid the natural healing process of body. Homeopathy involves the use of highly diluted preparations with similar therapeutic effect retained. Homeopaths have claimed that their method of preparing dilutions imparts qualities that retain the therapeutic effect.[1] Homeopathic medicinal products are used in other therapeutic approaches with a different methodological status, such as homotoxicology, isotherapy. Homeopathic medicines represent a significant part of medical economies and despite the growing use of homeopathic medicines worldwide, safety remains a major concern.[2] It is usually taken lightly on safety aspects of homeopathic medicines, as these are often highly diluted when administered. Various controversies dogged homeopathy

primarily that the high dilution of substance could reduce the likelihood of adverse effects.[2] There is lack of scientific reports available on safety data of homeopathic medicines. The various homeopathic medicines such as causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja are available in different formulations or preparations for the treatment of different ailments. These aforementioned selected homeopathic drugs have been studied in depth for the presence/or absence of any toxic substance.

Homeopathic causticum is a popular remedy made from the compound called potassium hydrate. As a homeopathic remedy, causticum has many potential health benefits. It is a key remedy for joint and arthritic conditions and skin problems like severe burns. Homeopaths also prescribe it for tremors and paralysis, urinary disorders, respiratory disorders, hemorrhoids, fibromyalgia and more.[3] Calcarea carbonica is obtained from the calcium in oyster shell. It is commonly used for backaches, joint pain, bone pain, bone growths, arthritis, rheumatoid arthritis pain, carpal tunnel syndrome or even bunions.[4] Medorrhinum is an effective homeopathic remedy produced from the bacterium *Neisseria gonorrhoeae*. The collection of bacterium was done from the urethral samples of a gonorrhoea male patient. It is persuasive homeopathic remedy to cure different ailments such as inflammatory (pelvic disorders) and in pain (ovarian and menstrual).[5] It is generally prescribed for different health settings of kidney, spines, mucous membranes and for nerves. Mercurius corrosive is ultra diluted homeopathic preparation prepared from chemical reaction involving mercuric chloride. It is potent in alleviating variant ulcers with most common ulcerative colitis and accompanying diarrhoea, bleeding, and pain.[6] Formica rufa is prepared from the red ants and mitigate gout, rheumatoid arthritis, nausea, headache and vertigo.[7] Silicea is also a form of the non-metallic element silicon or silicon dioxide as one of the major elements of the earth's crust and a vital constituent in plant structure. Homeopathic silicea is also used for eye, nose, throat, bone, joint, nerve system, and digestive problems. Silicea is especially useful for arthritis, knee inflammation of the bone, weak wrists, and subcutaneous nodules of hip joints when there is painful swelling.[8] Sulphur is an elementary substance, occurring in nature as a brittle crystalline solid, burning in the air with a blue flame. The reputation of Sulphur as a remedy is perhaps as old as medicine. The mineral Sulphur is found in every cell of the body. It is especially concentrated in the hair, skin and nails. It is an extremely important homeopathic remedy. It is used for conjunctivitis, eczema, colds, digestive disorders, nausea, constipation, hemorrhoids, diarrhea and shortness of breath.[9] Thuja occidentalis, also known as northern white-cedar or eastern arborvitae, it is an evergreen coniferous tree, in the cypress family Cupressaceae, which is native to eastern Canada and much of the north, central and upper Northeastern United States, but widely cultivated as an ornamental plant. Thuja is used for respiratory tract infections such as bronchitis, bacterial skin infections, and cold sores.[10] There are very few studies that have targeted the eventual benefice of these selected homeopathic drugs. The safety is the major concern in usage of these aforesaid homeopathic medicines for different ailments. The current study was devised to investigate the safe aspects of homeopathic drugs in acute and sub-acute toxicity study. In addition, the effect of these homeopathic medicines on vital organs is also taken into account.

Materials and Methods

Experimental Animals

Wistar rats weighing 150–250 g were obtained from the Central Animal Facility of AIIMS, New Delhi. The rats were housed individually under normal laboratory conditions with natural light-dark cycle and controlled temperature (20–25^{0}C) and humidity. The animals were acclimatized to the environment for a week prior to experimentation with free access to water and standard diet for rats. Homeopathic medicines were given orally, using feeding cannula, and were observed for incidence of mortality and sign of intoxication daily. Animals were housed in labelled cages and their fur was tagged with methylene blue for identification. The protocol was approved by the Institutional Animal Ethics Committee (983/IAEC/16). After approval, animals were taken, weighed and distributed randomly into appropriate groups for conducting the study. The experiments were carried out in the premises of the Animal House, Department of Pharmacology, AIIMS.

Chemical and Drugs

All the chemicals used were of analytical grade. The selected homeopathic drugs (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) were supplied by CCRH, Ministry of AYUSH, Govt. of India, New Delhi.

Dose Calculation of Study Drugs for Experimental Animals

Dose of homeopathic drugs were calculated as per advice of CCRH

Route of administration = per oral

Vehicle for administration = De-ionized water

Standard dose of 2000 µl/100g was considered for acute toxicity study (single bolus dose) and for sub-acute toxicity study, the dose used was ten times less than acute toxicity study and administered daily for 28 days (20 µl/100g body weight).

Experimental design: Toxicity studies

Acute-toxicity study

Evaluation of acute oral toxicity of all homeopathic medicines (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) was carried out according to the OECD guidelines for testing of chemicals-425 (OECD, 2001). Animals were divided into ten groups of homeopathic medicines. A limit test (2000 µl/100g body weight) was performed on female Wistar rats (150–250 g) from the breeding stock. All the animals were observed for behavioural changes and mortality till 14 days after administration of the dose.[11]

Sub-acute toxicity studies

Evaluation of 28-day oral toxicity study of homeopathic medicines was carried out according to the OECD guidelines for testing of chemicals-407 (OECD, 2008). Wistar rats (150–250 g) of both sexes, from our breeding stock were allocated into eleven groups (n = 5/sex/group). Group I received the normal control (1 ml/kg body weight, 1% saline) and group II-XI received Causticum, Calcarea, Medorrhinum Mercurius, Formica, Proteus, Silica, Placebo, Sulphur, Thuja respectively. Drug/vehicle was administered daily, 20 µl/100g orally for 28 days via oral gavage. Behavioural parameters, Body weight and any toxic signs were noted down on daily basis.[12]

Evaluation parameters

Biochemical Parameters: The different biochemical parameters were evaluated with the help of standard analytical kits from Erba Diagnostic Kits (Transasia Biomedical Ltd.) Mumbai, India according to manufacturer instructions. The biochemical analysis measured were triglycerides (TG), high density lipoprotein (HDL), blood glucose (GLU).

Clinical chemistry: Liver enzymes SGOT and SGPT and serum creatinine level were estimated in testing samples using kits from Span Diagnostic Labs, Karnataka, India.

Haematological parameters

The total counts of Red blood cell, white blood cells, platelets, haemoglobin, BT and CT were measured using haematological analyser (Sysmex XS-1000i). The analysed haematological parameters were RBC, WBC, HB, platelets, bleeding and clotting time.

Necropsy: After the experimental period, the rats were euthanized by cervical dislocation and subjected to gross necropsy and the findings were recorded.

Organ weights: After detailed gross necropsy examination, the following vital organs Liver, Kidney, Heart, Brain, testis and ovaries, were collected from each animal and the weights were recorded.

Histopathology: The following organs and tissue samples were collected from all the animals and preserved in 10% buffered neutral formalin. They were sliced adequately wherever necessary. After a minimum of 24 hr fixation, they were sampled and processed by conventional methods, paraffin blocks were made and 6 µm paraffin in sections was stained with haematoxylin and eosin. They were examined under a light microscope for changes in structure and the pictures taken with digital camera attached to the eyepiece of the light microscope. All deviations from normal histology were recorded and compared with corresponding controls- Liver, Kidney, Brain, Heart, testis and Ovaries.[13]

Statistical analysis

All data produced in this study were represented as Mean ± SEM (n=6). ANOVA was used to calculate the comparison between groups followed by Dunnett's multiple comparison tests. The statistical tools were performed by Graphpad Prism version 5.03, San Diego, CA, USA considering $p < 0.05$ to be statistically significant.

Results

Effect of homeopathic drugs on acute oral toxicity study

In acute toxicity study, we observed no significant deviation in behavioural patterns of all the experimental animals for study period of 14 days. There were no mortality or toxic signs

were observed in homeopathic medicines treated groups which indicate that oral LD_{50} was found to be greater than 2000 µl/100g body weight. The numbers of animals alive/tested were represented in Table 1.

Effect of homeopathic drugs on sub-acute oral toxicity study for 28 days

The homeopathic medicines were administered to experimental animals daily for a period of 28 days in accordance to OECD-407. We found that there was no statistical significance difference in behavioural parameters like walking, sleeping and eating pattern in all the experimental animals and we found no unusual activity among any group. We noted down that there was no sign of toxicity in experimental groups.

Body weight

No significant changes in body weights of homeopathic medicines treated rats were observed when compared with normal control group on day 7, 14, 21 and for 28 days. The mean change in body weight of normal control and treated groups in male (Fig. 1) and female (Fig. 2) Wistar rats was monitored and difference found among groups is non-significant statistically.

Relative organ weight

After daily administration of homeopathic medicines for 28 days, we observed that there were no significant changes in mean organ weight of different organs. No signs of Gastric ulceration or erosion were observed. Change in mean organ weight of different organs in control and treated groups (Data not shown).

Effect on haematological parameters

Table 1: Results of Acute Oral Toxicity of Test Compounds

S. No.	Groups	Days									
		1	2	3	4	5	6	7	8	9	10
		No. of Animals Alive/ Tested									
1	Causticum	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2	Calcerea	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3	Medorrhinum	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4	Mercurius	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
5	Formica	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
6	Proteus	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
7	Silica	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8	Placebo	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
9	Sulphur	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
10	Thuja	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Table 2: Effect of administering different homeopathic drugs on Hematological parameters in Wistar rats for period of 28 days (Male). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons.

Drug Treatment	Hematological parameters					
	RBC	WBC	Platelets	Hemoglobin	BT	CT
Normal Control	9.14 ± 0.22	15.86 ± 1.00	966.2 ± 8.29	14.14 ± 0.51	7.07 ± 0.04	1.73 ± 0.03
Causticum	9.02 ± 0.19	16.27 ± 1.41	971.2 ± 11.21	14.60 ± 0.32	7.02 ± 0.06	1.73 ± 0,02
Calcerea	9.06 ± 0.2	14.06 ± 0.95	977.6 ± 5.71	14.14 ± 0.56	7.02 ± 0.03	1.75 ± 0.03
Medorrhinum	8.90 ± 0.11	16.52 ± 1.30	977.0 ± 7.82	14.36 ± 0.56	7.05 ± 0.04	1.77 ± 0.04
Mercurius	9.26 ± 0.17	17.33 ± 1.45	984.6 ± 6.74	14.20 ± 0.5	7.07 ± 0.05	1.78 ± 0.01
Formica	9.32 ± 0.14	18.42 ± 1.19	978.6 ± 9.17	14.66 ± 0.35	7.04 ± 0.04	1.76 ± 0.03
Proteus	9.30 ± 0.18	18.90 ± 0.66	970.0 ± 7.99	14.16 ± 0.47	7.04 ± 0.04	1.77 ± 0.03
Silica	9.22 ± 0.13	18.87 ± 1.89	977.4 ± 10.92	14.00 ± 0.50	7.01 ± 0.05	1.75 ± 0.02
Placebo	9.28 ± 0.12	18.28 ± 1.17	975.0 ± 10.50	14.78 ± 0.58	7.04 ± 0.06	1.76 ± 0.04
Sulphur	9.54 ± 0.09	19.13 ± 1.95	970.0 ± 8.20	14.66 ± 0.29	7.03 ± 0.03	1.72 ± 0.04
Thuja	9.44 ± 0.12	18.06 ± 1.30	976.6 ± 10.03	14.06 ± 0.39	7.05 ± 0.06	1.74 ± 0.04

Haematological parameters (RBC, WBC, Platelets, Haemoglobin, BT, and CT) were analysed after daily administration for 28 days. All the haematological parameters of treatment groups were remained within physiological range and found no significant changes. The effect of haematological parameters of treatment groups in compared to normal group is represented in Table 2, 3. In male Wistar rats, no statistically significant ($p > 0.05$) difference was found between the groups and similar pattern was observed in female Wistar rats.

Biochemical analysis also did not produce any significant changes in biochemical parameters (Blood glucose, Serum creatinine, SGOT, SGPT, TG, HDL) of male (Table 4) and female (Table 5) rats as compared to those in normal control group. All animals survived until the scheduled necropsy and their physical and behavioural examinations did not reveal any treatment-related adverse effects. No statistical difference ($p > 0.05$) between groups in male and female animals was noticed that exhibits absence of toxic effects post homeopathic drugs administration.

Effect on biochemical parameters

Table 3: Effect of administering different homeopathic drugs on Hematological parameters in Wistar rats for period of 28 days (Female). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons.

Drug Treatment	Hematological parameters					
	RBC (million/mm^3)	WBC (thousand/µL)	Platelets (thousand/µL)	Hemoglobin (g/dl)	BT (min)	CT (min)
N. Control	8.26 ± 0.09	13.46 ± 1.01	37.88 ± 1.20	12.88 ± 0.46	6.58 ± 0.10	1.60 ± 0.03
Causticum	8.00 ± 0.14	13.06 ± 1.11	35.22 ± 1.31	13.70 ± 0.51	6.63 ± 0.10	1.60 ± 0.03
Calcerea	8.22 ± 0.14	14.21 ± 0.45	34.28 ± 1.20	13.30 ± 0.58	6.59 ± 0.10	1.60 ± 0.04
Medorrhinum	7.88 ± 0.13	12.71 ± 0.71	35.39 ± 1.43	13.06 ± 0.53	6.63 ± 0.06	1.61 ± 0.04
Mercurius	8.22 ± 0.15	14.40 ± 1.23	35.65 ± 1.35	13.46 ± 0.76	6.61 ± 0.05	1.63 ± 0.05
Formica	8.12 ± 0.19	13.39 ± 1.07	34.86 ± 1.12	13.36 ± 0.57	6.46 ± 0.09	1.62 ± 0.03
Proteus	8.36 ± 0.18	14.30 ± 0.88	32.43 ± 1.23	13.44 ± 0.59	6.60 ± 0.10	1.60 ± 0.04
Silica	7.94 ± 0.12	13.73 ± 1.12	33.86 ± 1.98	13.22 ± 0.76	6.58 ± 0.09	1.59 ± 0.04
Placebo	8.16 ± 0.20	14.07 ± 0.69	36.73 ± 1.76	13.88 ± 0.46	6.62 ± 0.07	1.61 ± 0.04
Sulphur	8.12 ± 0.21	14.50 ± 0.82	38.91 ± 1.65	13.42 ± 0.59	6.67 ± 0.07	1.58 ± 0.06
Thuja	8.44 ± 0.13	13.96 ± 1.31	37.34 ± 1.55	13.58 ± 0.75	6.59 ± 0.08	1.61 ± 0.05

Table 4: Effect of administering different homeopathic drugs on biochemical parameters (SGOT, SGPT, blood glucose, serum creatinine, triglycerides, HDL) of Wistar rats for period of 28 days (Male). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons.

Drug Treatment	Biochemical parameters					
	Blood Glucose	Serum Creatinine	SGOT	SGPT	TG	HDL
Normal Control	103.8 ± 1.49	0.46 ± 0.06	116.0 ± 1.30	45.60 ± 1.80	56.80 ± 2.63	43.20 ± 1.46
Causticum	104.2 ± 1.93	0.42 ± 0.05	115.4 ± 2.46	45.80 ± 1.28	59.00 ± 1.37	43.40 ± 1.56
Calcerea	1102 ± 2.12	0.44 ± 0.07	117.2 ± 2.70	43.40 ± 1.43	59.40 ± 2.5	44.00 ± 1.30
Medorrhinum	103.6 ± 1.77	0.48 ± 0.07	116.8 ± 1.06	46.00 ± 2.73	58.40 ± 1.5	42.00 ± 1.22
Mercurius	104.0 ± 1.81	0.42 ± 0.03	115.6 ± 2.06	46.40 ± 1.80	57.80 ± 2.13	44.80 ± 1.15
Formica	102.8 ± 1.35	0.46 ± 0.05	116.6 ± 1.60	45.20 ± 2.03	59.80 ± 1.53	44.00 ± 1.15
Proteus	105.0 ± 2.07	0.48 ± 0.07	114.4 ± 1.86	47.00 ± 1.51	59.00 ± 2.60	43.80 ± 1.65
Silica	103.2 ± 1.59	0.48 ± 0.08	118.6 ± 1.03	45.80 ± 1.82	60.60 ± 1.20	43.20 ± 1.39
Placebo	100.4 ± 1.16	0.46 ± 0.05	116.2 ± 1.15	44.60 ± 2.42	60.40 ± 2.15	45.00 ± 1.22
Sulphur	101.0 ± 1.22	0.42 ± 0.05	114.8 ± 2.2	44.00 ± 1.51	59.00 ± 1.64	44.20 ± 1.65
Thuja	101.6 ± 1.88	0.50 ± 0.07	114.2 ± 1.42	47.00 ± 1.58	60.20 ± 1.46	45.60 ± 1.03

Histopathological evaluation

No pathological changes were observed in histological section of vital organs measured i.e heart, kidney, liver, testis, ovaries and brain of homeopathic drugs treated (Figs. 3, 4, 5) rats as compared to normal control animals. Morphological changes did not reveal cell degeneration or inflammatory cell infiltration in any of the tested homeopathic drugs. The histological sections of liver showed regular arrangement of cells and sections of kidney also showed normal architecture of glomerulus. Thereby no changes were observed at cellular level of these vital organs. Pictomicrographs of histology section of kidney, liver, heart, brain, testis (male) and ovaries (female) of different homeopathic drugs was depicted in Figs. 3, 4 and 5.

Table 5: Effect of administering different homeopathic drugs on biochemical parameters (SGOT, SGPT, blood glucose, serum creatinine, triglycerides, HDL) of Wistar rats for period of 28 days (Female). All values are Mean ± SE. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons. $p > 0.05$ represents non-significant

Drug Treatment	Biochemical parameters					
	Blood Glucose	Serum Creatinine	SGOT	SGPT	TG	HDL
Normal Control	101.8 ± 1.42	0.40 ± 0.03	124.2 ± 1.28	45.80 ± 1.39	52.80 ± 2.43	41.60 ± 1.20
Causticum	101.2 ± 1.15	0.42 ± 0.05	125.6 ± 1.99	42.40 ± 1.63	56.00 ± 2.55	40.00 ± 1.41
Calcerea	100.6 ± 1.91	0.44 ± 0.05	126.2 ± 2.17	44.20 ± 1.93	56.40 ± 3.07	42.80 ± 1.56
Medorrhinum	101.2 ± 1.56	0.46 ± 0.05	124.6 ± 2.04	45.00 ± 2.09	54.20 ± 2.08	43.80 ± 2.22
Mercurius	100.4 ± 1.50	0.44 ± 0.06	125.6 ± 2.13	46.40 ± 2.11	56.20 ± 2.87	43.00 ± 1.64
Formica	99.80 ± 1.46	0.40 ± 0.05	125.4 ± 1.43	43.20 ± 1.82	50.80 ± 2.88	40.40 ± 1.28
Proteus	100.8 ± 1.15	0.46 ± 0.05	126 ± 1.51	44.40 ± 1.96	55.40 ± 2.42	43.00 ± 1.51
Silica	101.8 ± 1.46	0.44 ± 0.05	126.8 ± 1.49	45.60 ± 1.50	52.80 ± 3.02	43.40 ± 1.36
Placebo	100.6 ± 1.77	0.46 ± 0.04	123.6 ± 2.29	43.40 ± 1.43	52.60 ± 2.61	42.00 ± 1.81
Sulphur	101.0 ± 1.22	0.42 ± 0.05	125 ± 1.34	46.60 ± 1.63	50.20 ± 1.98	42.20 ± 1.39
Thuja	102.0 ± 1.41	0.42 ± 0.03	126.6 ± 1.20	46.00 ± 2.12	54.20 ± 2.39	40.80 ± 1.15

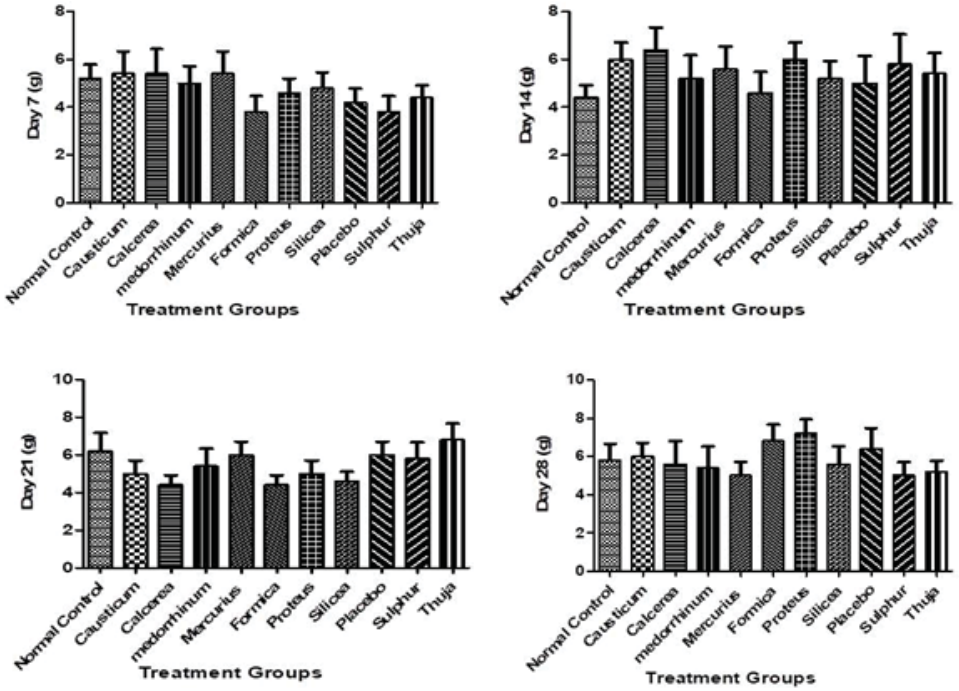

Fig. 1: Effect of administering different homeopathic drugs on mean change in body weight of rat over a period of 28 days (Male). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons. $p > 0.05$ represents non-significant.

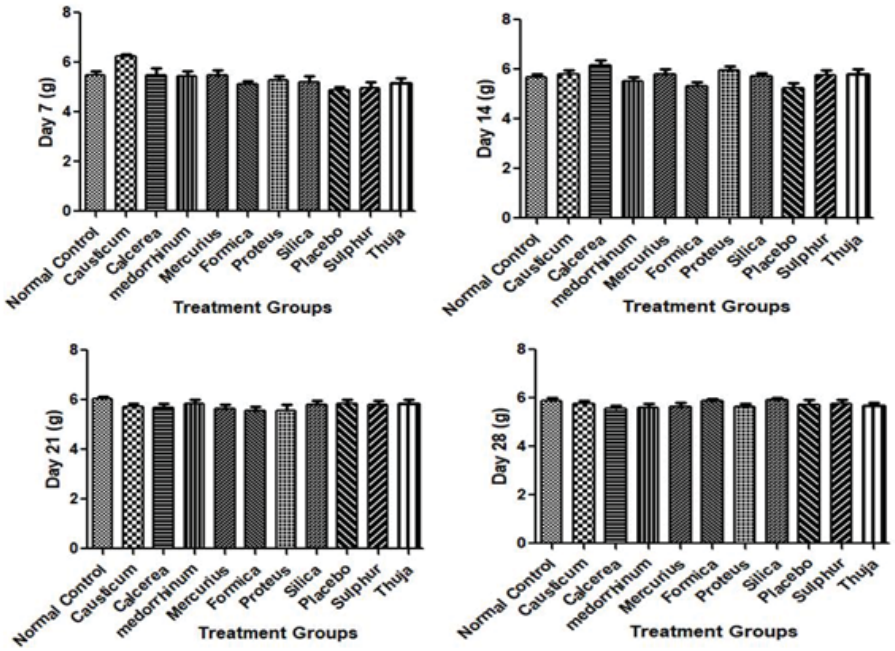

Fig. 2: Effect of administering different homeopathic drugs on body weight of rat on day 7 over a period of 28 days (Female). All values are Mean ± SEM. Statistical mean change in analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons. $p > 0.05$ represents non-significant.

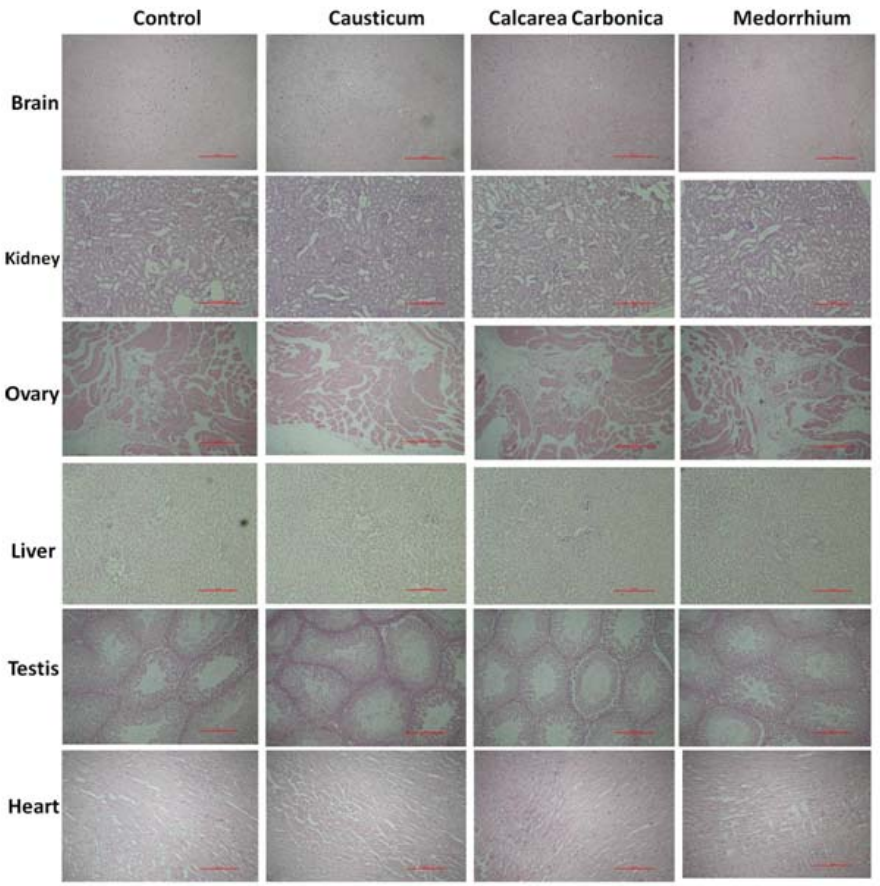

Fig. 3: Effect of normal control and different homeopathic drugs (Causticum, Calcerea carbonica, Medorrhinum) on microscopic examination of different vital organs (Brain, liver, kidney, heart, testis, ovaries) stained by H & E. (Pictographs were randomly taken from group of five rats) Micrographs

were taken as 10x, scale bar 100 µm.

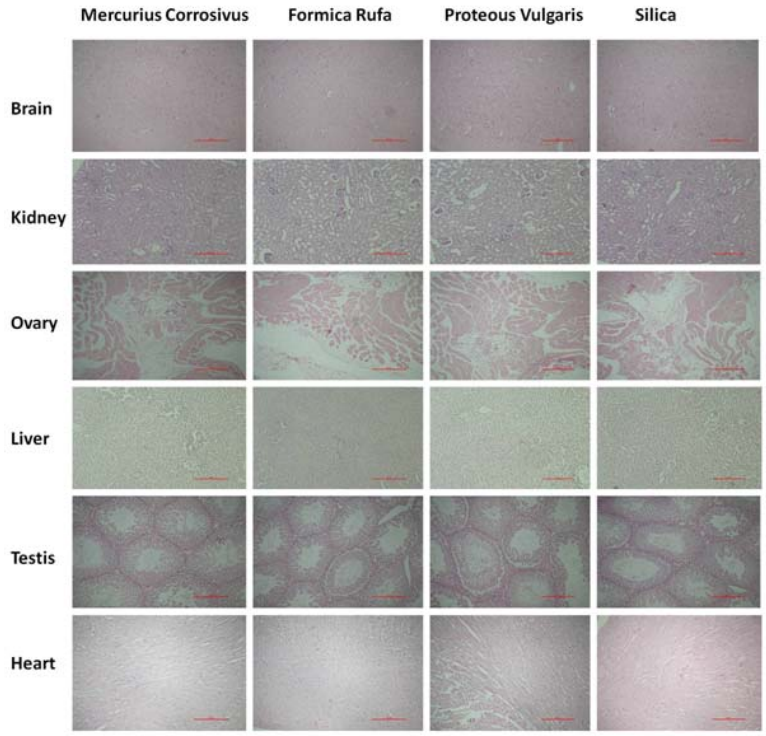

Fig. 4: Effect of different homeopathic drugs (Mercurius Corrosives, Formica Rufa, Proteous Vulgaris, silica) on microscopic examination of different vital organs (Brain, liver, kidney, heart, testis, ovaries) stained by H & E. (Pictographs were randomly taken from group of five rats) Micrographs were taken as 10x, scale bar 100 µm.

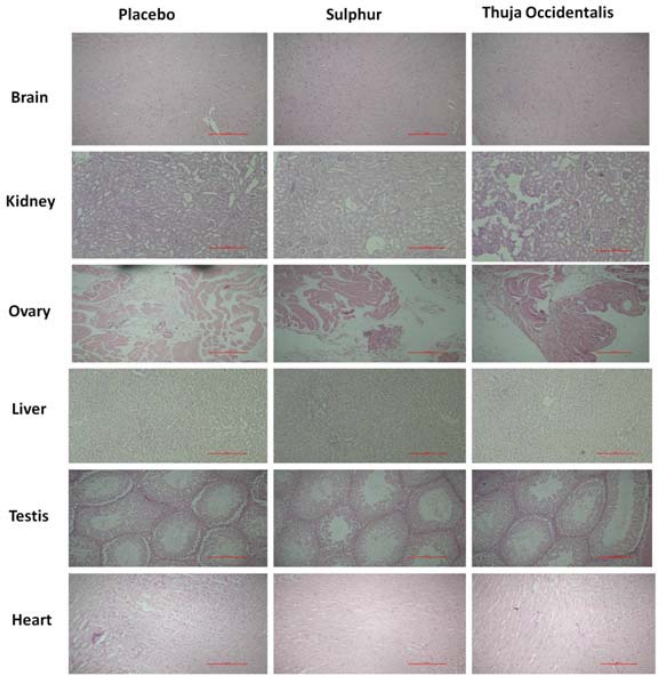

Figure 5: Effect of different homeopathic drugs (Placebo, Sulphur, Thuja Occidentalis) on microscopic examination of different vital organs (Brain, liver, kidney, heart, testis, ovaries) stained by H & E. (Pictographs were randomly taken from group of five rats) Micrographs were taken as 10x, scale bar 100 µm.

Discussion

In the current study, the toxicity profile of selected ultra-diluted preparations was evaluated in accordance to OECD guidelines. As we know that there is a myth in the society that the homeopathic drugs are generally safe. The principle of homeopathic preparations works with the involvement of high dilution factor. However, there is lack of evidences present on safety aspects of homeopathic preparations. Therefore, to scientifically evaluate the toxicity of selected homeopathic drugs was undertaken and experimental studies were carried out.

Acute and sub-acute toxicity study protocol was in corroboration to OECD 425 and 407 respectively.[11,12] In acute toxicity study, the selected homeopathic drugs administered daily up to dose level of 2000 µl/100g body weight (ten times more than effective dose; 20 µl) to the treatment groups and kept in observation for 14 days. We observed the mortality of tested animals for study period and found that the LD50 was found to be greater than 2000 µl/100g body weight in Wistar rats. We examined that up to this dose, mortality in treatment groups was not found and also there were no significant changes in body weights. Previous reports suggest that change in body weight is an imperative marker to study any toxic effects to the individual.[14] Sub-acute toxicity study was carried out for a study period of 28 days to investigate the effect of homeopathic drugs for prolonged period in tested animals. The dose taken for these studies was ten times lower than the dose used in acute toxicity study. Therefore, in our current work, we devised to investigate the effect of homeopathic drugs on body weight, biochemical, haematological and histopathological analysis. The body weight of all the animals were measured at regular time interval i.e. on day 7, 14, 21 and 28. In same manner organ weight of vital organs (liver, kidney, heart, brain, testis and ovaries) were also measured and found to be constant throughout the study period. The findings are in harmony with Ghosh et al. wherein the same pattern of observations was found in organ weight.[15] Meanwhile the behavioural parameters like sleeping, walking and eating were also monitored during the study period and found no abnormality when compared to normal control group.

Sub-acute toxicity study is effective in assessing the effect of homeopathic drugs on target organ, on haematological and biochemical alterations, as these effects are usually not observable in acute toxicity test. Biochemical parameters are the key diagnostic markers to examine any abnormal finding in clinical settings. Biochemical parameters (Blood glucose, Serum creatinine, SGOT, SGPT) were examined in this study. Serum liver function tests provide the information regarding the status of liver. Liver enzymes SGOT, SGPT described the cellular integrity of liver and on the contrary albumin and protein level determine the functionality of liver. As we know that SGOT and SGPT are the key enzymes principally produced by liver cells and any alteration or abnormality to liver, leads to the increase in serum level of these enzymes.[15] Augmentation on these serum enzymes predicts the sign of hepatocellular toxicity, whereas decrease in these leads to the enzyme inhibition. SGPT is the most sensitive marker of liver damage or toxicity. We did not observe any variation in SGPT and SGOT level when compared with normal control animals. Blood glucose is the imperative marker to study the diabetes and prediabetes stage or variation in energy metabolism.[16,17] Blood glucose level showed no significant ($p > 0.05$) changes in treatment groups in comparison to normal control animals. Alteration in hematopoietic is one of the critical steps to determine the drug toxicity. Parameters such as RBC, WBC, Platelets, Hb, BT and CT were studied and found that these were lie within the respective normal range in homeopathic groups which was in comparison to normal control estimates. The gross histology of different organs was performed by haematoxylin and eosin staining. The brain section of Wistar rats in homeopathic drugs showed a symmetric pattern in left and right hemispheres.

The regions of Cerebrum, cerebellum and grey matter revealed healthy Astrocytic cells with prominent nuclei and it is in close proximity to neurons in all tested homeopathic drugs. As stated earlier the microscopic examination of heart section of nine homeopathic drug demonstrated no abnormality in architecture of cardiomyocyte. The normal architecture of cardiac myocytes was maintained with absence of necrosis, vacoulation or infiltration of mononuclear or inflammatory cells. As shown in figures 3,4,5. Histological analysis of liver sections showed a network of hepatocytes that are arranged in single cell thick plates separated by vascular sinusoids. Absence of lipid accumulation, focal inflammation was observed rendering homeopathic drugs safe for hepatic functioning. As depicted in figures 3,4,5. Histology of testis showed well organised germinal epithelium in normal and homeopathic treated groups. No observable difference in seminiferous epithelium of tubules was seemed with single layer of sertoli cells. These depicted no sign of inflammatory

secretions implying no toxicity due to homeopathic drugs on male reproductive organ. Gross histology of ovaries comprises of cuboidal epithelial cells in continuation with peritoneal mesothelium. There was no variation in ovary histology pertaining to homeopathic drug administration. Homeopathic drugs maintain normal homeostasis and thereby did not produce any alteration in microscopic examination. The gross necropsy analysis of various organs did not portray any legends, oedema or observable abnormalities. There we can say that homeopathic drugs (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) did not exposed any treatment related adverse effect on various organs.

Conclusion

Acute and sub-acute toxicity study is imperative to assess safety as well as in translation research. LD50 of nine homeopathic drugs used in the study obtained from different sources, was found to be greater than 2000 µl/100g body weight. Selected homeopathic drugs are devoid of toxicity on biochemical, haematological in 28 days repeated sub-acute toxicity study. The findings suggest that homeopathic drugs have NOAEL (no observed adverse effect level) at dose of 20 µl/100g body weight. Further elaborative experimental analysis of chronic toxicity study is imperative to support the findings.

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Conflict of Interest: There was no conflict of interest among authors and co-authors.

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Original Article

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Detection of Nerve Agent Metabolites in Plasma and Urine by ^{31}P NMR Spectroscopy

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Abstract

A simple robust ^{31}P nuclear systems based NMR method is presented for the detection of alkyl methylphosphonic acids; metabolites of three organophosphorus nerve agents (GB or Sarin, GD or Soman, and GF or Cyclosarin) in the biological spiked samples of plasma and urine. The method gives linear response (correlation coefficients ~0.89) over the concentration range 10 µg/ml to 300 µg/ml in urine and plasma considering phosphorus nucleus. The quality control data demonstrated accurate identification of biomarkers of nerve agents at low concentration. NMR based method provides direct analysis of Biological samples without any sample pretreatment or sample preparation and sample tempering.

Keywords: Isopropyl methylphosphonate; Hydrolysis; 1H; 31P; NMR; Plasma; Urine.

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Introduction

Owing to the simple synthesis of nerve agents, these might be considered as one of the possible terrorist weapons in the present scenario. An increasing use of these agents has posed new challenges for the retrospective detection and identification of these chemicals in the environmental and biological samples.[1] The simplest way to decide their alleged use is detection of presence of their metabolites in the environmental and biological samples. G-agents were first introduced by Germany when Gerhard Schrader discovered the sarin, later GF and tabun were developed. Former Soviet Union and Britain developed VR (Russian V series nerve agents similar to VX also called Novichok agents) and VX (O-ethyl S-[2-ethyl] methylphosphonothioate) respectively.[2] So far besides the sulfur mustard other CW agents like nerve agents have also been used in the wars and terrorist attacks. SM (HD) and nerve agents were used by Iraq in Iraq-Iran war during 1983–1988, and against Kurdish people in 1988. Sarin and VX were also used by terrorist group in Japan in 1994 and 1995.[3] Series of reports reveal that Chemical weapons were also used in Syrian civil war during 2012 to 2017.[4,5,6]

Nerve agents are neurotoxins. These agents bind with serine moiety of Acetylcholinesterase enzyme in the nerve synapses and inhibit the hydrolysis of neurotransmitter acetylcholine. Due to this, excessive accumulation of acetylcholine occurs in nerve synapses, which causes over stimulation of nerves and ultimately paralysed various muscles. In later stage usually paralysis of respiration leads to death.[7]

As far as their detection is concerned, Nerve agents exposure is detected in the samples either

by the direct presence of intact agent, or by their metabolites or by their adducts formed with tyrosine moiety of albumin protein or through the development of seizure related brain damages. Their presence in the samples depends upon the exposure concentration and time of sample collection of post exposure.[8]

It has been observed that various Mass based hyphenated analytical techniques like LC/MS/MS, GC/Ms [8] etc have been used for the analysis of biomarkers of nerve agents in the environmental and biological samples but less emphasis is been given on NMR based methods in detection field, owing to less sensitivity of NMR.[9] Mass spectrometric techniques are by far the most popular and sensitive, but these techniques require lengthy sample preparation methods like derivatization and extraction methods, while NMR analysis offers a direct, multinuclear and nondestructive method of analysis. So far Nuclear Magnetic Resonance Spectroscopy (NMR) is recognized as tool for characterization of molecules, kinetic studies of chemical reactions, metabolomics and for the detection of a wide range of small endogenous or exogenous molecules in the complex biofluids such as urine, plasma and tissues. Therefore, here NMR is utilized for direct monitoring of the hydrolysed products, without any issues associated with extraction, derivatization, chemical compatibility, background effect, manipulation of samples, and solvent effect.[7]

In this paper NMR based method is demonstrated for the detection of metabolites in urine (human) and serum (rat) spiked samples by the effective use of ^{31}P NMR active nucleus. Detection method is optimized by performing the number of experiments with various concentrations of hydrolysed products in buffered solutions at various pHs.

Materials and Methods

Urine samples were obtained and analysed using an IRB protocol approved for obtaining random healthy volunteers. Urine specimens were collected from eight individuals asked to join the study in a random anonymous fashion, to prevent any sort of bias.

Blood samples of male Harlan Sprague-Dawley rats were collected into heparinised tubes *via* the retro-orbital venous plexus using anesthesia. The plasma was immediately separated from red cells by centrifugation (2000 rpm, 20 min). Both urine and plasma samples were stored at - 80°C prior to analysis. This was done in accordance with the Helsinki Declaration of 1983. NMR solvent D_2O, Tritisol buffer capsules were procured from Merck. Deionized water was procured from a Millipore Milli-Q filtration system. Chemicals required for synthesis like thionyl chloride, triethyl amine, isopropyl alcohol, pinacolyl alcohol, cyclohexyl alcohol and Dimethyl methylphosphonate etc. were purchased from Merck and Aldrich.

NMR Spectroscopy

The internal standards TSP at 0 ppm (Trimethylsilylpropanoic acid) and TMP (Trimethyl phosphate) at 2.5 ppm were used for recording the ^{1}H and ^{31}P NMR spectra of standard samples of CW (Chemical warfare) agents and their metabolites respectively. Minimum detection limit of phosphorus nucleus was evaluated by setting the chemical shift scale externally using 85% phosphoric acid in D_2O at 0.00 ppm. Urine and plasma samples were also screened using external standard for ^{31}P nucleus. Thawed samples were centrifuged at 20,000 rpm, 4°C, for 20 min. 200 µl of D_2O was added to 400 µl of urine samples and 400 µl of D_2O was added to 200 µl of plasma samples for recording of spectra. The 1D ^{1}H and ^{31}P spectra were recorded at magnetic field 400.13 MHz and 161.97 MHz respectively on a Bruker DPX-400 MHz spectrometer at 300 K temperature using a standard pulse sequence comprising 5 µs pulse width with 2 µs delay and a pulse power of 3 db. The water resonance was suppressed by presaturation experiment using relaxation delay (2s) and 20µs power switching for irradiation delay for recording ^{1}H NMR of standard authentic samples and urine samples in water. A total of 64 FIDs were accumulated into 64k data points over a spectral width of 12019 Hz with 8 dummy scans. A line-broadening factor of 0.3 Hz was applied before Fourier transformation. Chemical shifts were referenced internally to the CH_3 signal of endogenous lactate doublet at 1.33 ppm in plasma sample. The creatinine singlet at 3.06 was taken as the reference for spectra of urine samples. ^{31}P NMR spectra of both the samples were recorded using 5.40 µs pulse width, 9 sec. delay, 64k FID data points, 32 k spectral data points, 64935.06 Hz sweep width with 1 Hz line broadening. For urine samples, a standard 1D spectrum with selective irradiation of the water resonance, with relaxation delay (RD), 2s and mixing time (tm), 0.1s was acquired. Additionally, for the plasma samples, a spin-echo (CPMG) spectrum acquired with a total echo time of 608 ms.

To obtain the accurate results in quantitative NMR, 90° pulse calibration and pulse delay were

optimized to obtain the maximum recovery of magnetization.

Results and Discussion

Calibration Curve and Minimum detection limit

In the first set of experiments a concentration gradient consisting of seven solutions of IMPA (Isopropyl methylphosphonate) was prepared in urine containing buffer solution in concentration range of 5 µg/mL to 320 µg/mL for detector response calibration. Calibration curve was plotted between different concentrations of IMPA and signal height of ^{31}P nucleus. A straight line curve was obtained which represents linear detector response.

In the second set of experiments urine and human serum albumin solution (prepared in water), were prepared for elucidation of minimum detection limit of IMPA using ^{31}P NMR at neutral pH in D_2O. Here in place of plasma sample HBA was taken for experiment. Series of samples of IMPA with various concentrations ranging from 0.0 µg/mL (control), 1 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, 30µg/mL and 60 µg/mL were prepared from the stalk solution of 200 ppm. The minimum detection limit was found 20 ppm and 30 ppm in plasma and urine respectively in D_2O solvent at neutral pH using ^{31}P nucleus. It was observed that IMPA present minimum detection limit 8 µg/ml in $CDCl_3$. Fig. 1 Shows the structures of organophosphorus CW agents and their metabolites.

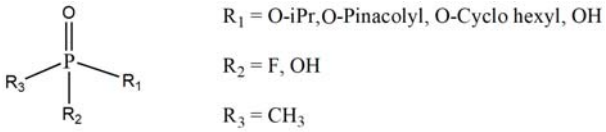
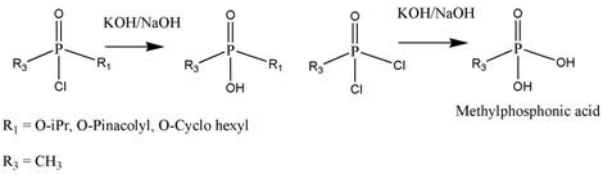


Fig. 1: General Structure of CW agents

Synthesis and NMR analysis of CW agents and metabolites

Nerve agents were synthesized by reported procedure[10] and their hydrolysed products were also prepared by the reaction of 30% NaOH (alkaline hydrolysis) as per the shown scheme 1. The methylphosphonic acid is synthesized directly from methylphosphonic dichloride using 30% NaOH solution. The analytes/metabolites those were aimed to detect using NMR analysis are: IMPA (Isopropyl methylphosphonic acid) of GB (Isopropyl methylphosphonofluoridate), PMPA (Pinacolyl methylphosphonic acid) of GD (Pinacolyl methylphosphonofluoridate) and CMPA (Cyclohexyl methylphosphonic acid) of GF-(Cyclohexyl methylphosphonofluoridate). The complete ^{1}H, ^{13}C, ^{31}P and ^{19}F chemical shifts values with coupling constant values for all the chemicals (nerve agents and hydrolysed products of nerve agents) were interpreted. There is great impact of polarity of Solvents used for analysis and pH of solution on the chemical shifts of the Phosphorus acids. Therefore the chemical shift of all the hydrolysed products were observed in different solvents $CDCl_3$, D_2O (pH= 2, 7 and 14) for phosphorus nucleus. These values are shown in the table 1.



Scheme 1: Hydrolysis of Nerve Agents

Hydrolysis of sarin into IMPA and MPA at various pHs

To monitor the hydrolysis reaction of sarin in NMR tube, Tritisol buffer capsules of pH 2, 7 and 14 were used to prepare solutions in water. Equal volumes of the sarin stock solution and buffer were added to NMR tubes along with deuterated water and vortexed. The final sarin concentration was 100 µg/ml in 0.5 M buffer. The chemical shift for ^{31}P signal of sarin was appeared as doublet at 30.891/37.344 ppm in D_2O solvent, because of coupling of phosphorus with fluorine. Analysis was performed within five minutes of sample preparation to observe the degradation path of sarin. The IMPA and MPA signals were observed at 31.9 and 31.4 ppm respectively at 1 pH after hydrolysis. Chemical hydrolysis of sarin into IMPA was rapid at pH 14, and slow at pH 1 and slowest at pH 7.0, reached 95% hydrolysis in about 24 hours. Methyl phosphonic acid was not found even after 2 days at pH 7 and 1. It was started forming in the basic solution after 08 Hrs, but complete hydrolysis into methyl phosphonic acid was not observed. Only 20% MPA was formed. The hydrolysis profile of sarin in plasma is shown in figure.

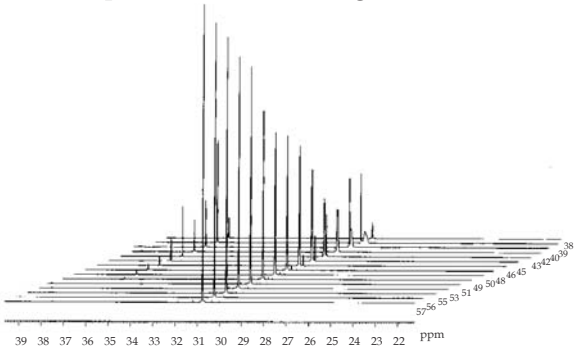


Fig. 2: Hydrolysis profile of Sarin

^{31}P NMR analysis of Sarin in Plasma and Urine

Sarin was taken in Plasma and Urine and solutions were mixed and kept under ambient temperature. The phosphorus NMR spectra was recorded after ten minutes which showed ^{31}P NMR chemical shift values for IMPA at 25.28 ppm in plasma (Fig 3) and 25.39 ppm in urine at 9 pH in D_2O solvent. Sarin hydrolysis was slow in the plasma and urine at neutral pH and took 48 hours for complete disappearance.

It has been observed that nerve agents produce characteristic signals as a metabolic response in proton and phosphorus NMR spectra with unique peak splitting information in the distinct chemical shift region. This factor helps in unambiguous detection of hydrolysed products in biological samples using ^{1}H NMR at high concentration of nerve agents.

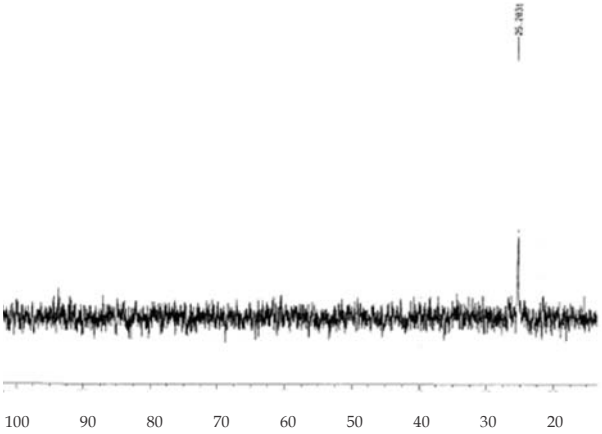


Fig. 3: ^{31}P NMR Spectrum of Isopropyl methyl phosphonic acid in Plasma.

However because of the low concentration of hydrolysed products of nerve agents, signals of

Table 1: ^{1}H, ^{31}P, ^{13}C and ^{19}F NMR Data of CW Agents and its Hydrolysed Products

S. No.	Name of the Chemicals	^{31}P{^{1}H} NMR δ solvent	^{19}F δ* *J**	^{1}H NMR δ	^{13}C NMR δ
	Isopropyl methylphosphono fluoridate	29.52 Doublet ($CDCl_3$)	-57.38 1046.4Hz	1.60(dd,3H,CH_3), ($^{2}J_{H-P}$ =18.7) ($^{3}J_{H-F}$=5.6 Hz); 1.36-1-39 (m,6H,-CH_3); 4.87 (m,H,CH)	11.06 (dd,C,CH_3-P), (J_{P-C} = 150); 71.50 (dd,C,CH-P), $^{2}J_{H-P}$=8) 23.84 (DD,3C, CH_3) ($^{3}J_{P-C}$= 4.49, $^{3}J_{F-C}$=2.7); 22.91(d,1C,-CH_3) (J_{F-C}=3.7)
	Isopropyl methylphos phonate	25.64(pH14), (D_2O), 27.18(DMSO), 30.56 (pH 1), (D_2O)	0	1.32(d,3H,CH_3), 4.69 (m,H,-CH); 1.32 (d,6H,CH_3)	27.92(d,C,CH_3), 76.23 (CH); 26.15 (CH_3)
	Methylphosphonic acid	20.32(pH14), (D_2O), 26.8(DMSO), 29.31 (pH 1), (D_2O)	0	1.27(d,3H,CH_3), ($^{2}J_{H-P}$=17.7)	13.9(d,C,CH_3), (J_{H-P}=139)
	Pinacolylmethylphosphono fluoridate	27.92, 29.16 ($CDCl_3$)	-58.26, -55.39 1047.5Hz	1.62, 1.64 (dd,3H,CH_3), ($^{2}J_{H-P}$=18.7) ($^{3}J_{H-F}$=7.6 Hz);4.387, 4.41(m,H,CH); ($^{3}J_{H-F}$=7.6, 8.9 Hz);1.32, 1.33 CH_3,($^{3}J_{H-F}$=0.6 Hz);0.98 (S,9H, CH_3)	10,05, 10.64 (CH3-P), (dd,C,CH_3-P), (J_{P-C} = 150);84.04, 83.75 (dd,C,CH-P), 16.60 (CH_3-CH), 25.4, 26.1(CH_3)$_3$
	Pinacolyl methylphosphonate	26.15 (pH 14), (D_2O), 28.31(DMSO), 32.26 (pH 1), (D_2O)	–	1.41 (d,3H,CH_3), ($^{2}J_{H-P}$=6.7),4.38(m,H,CH);1.327 CH_3, 0.91 (s,9H, CH_3)	13.01 (d,3H,CH_3), ($^{2}J_{H=}$138), 81,CH, 16.4CH-CH_3, 25.83, (CH_3)$_3$, 33.97 C-(CH_3)$_3$
	Cyclo hexyl methylphosphono fluoridate	30.2 ($CDCl_3$)	-56.9 1046.7Hz	1.63(dd,3H,CH_3), ($^{2}J_{H-P}$=18.7) ($^{3}J_{H-F}$=5.8 Hz); 4.62(m,H,-CH) ; 1.69-2.03(m,4H,CH_2-(CH) CH_2) ($^{3}J_{H-H}$=7.2,7.4); 1.16-1.64(m,6H,-CH_2-CH_2-CH_2)	10.5(dd,1C,CH_3), (J_{P-C}=147, $^{2}J_{P-F}$=28.1; 77.48(d,1C,-CH); 33.45, 33.56(CH_2-CH-CH_2),23.47, 23.49 (CH_2-CH_2-CH_2), 24.97(CH_2)
	Cyclohexyl methyl phosphonate	24.56 (pH 14), (D_2O), 32.28 (pH 1), (D_2O)	–	1.41(dd,3H,CH_3), ($^{2}J_{H-P}$=17.7); 4.52(m,H,-CH) ; 1.19-195 {m,10H, (CH_2-(CH)-CH_2)$_2$}	12.56(dd,1C,CH_3), (J_{P-C}=149); 76.48(d,1C,-CH); 33.84, (CH_2-CH-CH_2),23.87 (CH_2-CH_2-CH_2), 26.47(-CH_2-)

* δ = Chemical Shift, J = Coupling Constant

interest are obscured by signals of metabolites present in biological samples in proton NMR. Therefore detection of metabolites using Phosphorus nucleus becomes significant at low concentration and present superiority of ^{31}P over the ^{1}H, in which no other signals of plasma/urine metabolites appear in the region of interest and chances of overlapping of signals are also disappeared.

Conclusion

The aim of the study is optimization of simple and robust NMR based analytical method for the detection of metabolites of nerve agents in biological samples plasma and urine. In this method no sample pretreatment or sample manipulation is required, as it is required in other spectroscopic analysis. Studies performed on the chemical inter conversion of nerve agents showed that hydrolysis is rapid at alkaline pH, slow and less complete at neutral pH and slowest at acidic pH. NMR is an ideal system for direct analysis of metabolites.

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Caution: Nerve agents are extremely toxic in nature. During Synthesis full extra precautions must be taken care of. Proper protective gears and detectors should be used for analysis and during synthesis. Washing of glassware using 40% NaOH is always recommended. Before synthesis operating and synthetic procedures are advised to be study carefully.

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Original Article

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Uncertanity of Measurement During Estimation of 23 Organophosphorus Pesticides Residue Present in Bottle Gourd

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Abstract

The study presents the assessment of uncertainty calculation generated, within the analysis of selected 23 organophosphrus pesticides residues of bottle guard. The samples were prepared by using a modified quick, easy, cheap, effective, rugged and safe (QuEChERS) analytical protocol. Multiresidue method used for analysis of samples consisted of (i) acetonitrile extraction, (ii) PSA/C18 clean-up and (iii) identification/quantification of residues by GC utilizing either (nitrogen–phosphorus) or mass-selective detectors (quadrupole analyzer) were evaluated. Major sources like weighing of standard, purity of certified reference material, precision study i.e repeatability and standard solution preparation for calculation of uncertainty of method, was considered. Identification of uncertainty sources, quantification of uncertainty sources and calculation of combined uncertainty are steps for calculation of uncertainty. All the individual uncertainty calculated are combined, later converted to expanded uncertainty.

Keywords: Bottle guard; Uncertanity; Combined Uncertanity; Expanded Uncertanity; Pesticide.

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Inroduction

Estimated uncertainty of measurement is an integral part of analytical results. This paper focuses on major sources of errors concern with pesticide residue analysis. The sampling, sample processing and analysis influence the uncertainty and accuracy of analytical data. Their combined effects should be considered in deciding on the reliability of the results. (Stanisław Walorczyk, March 2014). The estimation of the uncertainty for any analytical methods is necessary for establishing the comparability of results. Multiresidue analytical methods lack very often of information about uncertainty of results, when results are compared with maximum residue levels (MRL) established by regulations. Identification and estimation of each uncertainty source allows laboratories to establish the accuracy of results and to balance with time-consuming and costs (L.Cuadros-Rodrıíguez, et al., 2002).

Uncertainty associated with analytical results make available as per ISO/IEC 17025 accrediated laboratories (ISO/IEC 2005). To calculate uncertainty, firstly measurand is specified, possible uncertainty sources are identified and quantified, finally combined uncertainty is calculated (EURACHEM/CITAC, 2000). Estimating the uncertainty for analytical measurements, basically two methods bottom-up and top-down methods

are applied. In the bottom-up method, analytical procedures are divided into individual components or steps. Their standard uncertainties are estimated and summed up together to form the combined uncertainty. Codex Committees on Methods of Analysis and Sampling (CCMAS) are working on the development of guidelines for estimation and interpretation of uncertainty of measurement results (CAC, 2010).

Materials and Methods

Total 23 Organophosphorus pesticides i.e Anilophos, Chlorfenvinfos, Chlorpyrifos, Chlorpyrifos-methyl, Dichlorvos, Ethion, Malathion, Parathion methyl, Monocrotophos, Phorate, Profenofos, Quinolphos, Trizophos, Fenitrothion, Phosalone, Paraxon-methyl, Fenamiphos, Edfinphos, Dimetoate, Diazinon, Fenthion, Parathion and Phosphomidon were taken for study. All pesticides taken were good sensitive for GC-FPD detector. In todays contrast pesticides are frequently used by farmers to protect their crops from insects, pests and weeds. Among various crops, vegetables are highly sprayed by pesticides. All pesticides standard of higher purity were procured from Sigma Aldrich (Germany). For extraction of vegetables from bottle gourd acetonitrile solvent was used. For moisture removal from sample, sodium sulphate anhydrous and magnesium sulphate anhydrous were used. For cleaning the sample the samples Primary Secondary Amine (40 µm, Bondesil) and C-18 silica sorbent were used. All the chemicals and solvents used during analysis were purchased from Sigma Aldrich and Merck Germany. The use of high purity reagents and solvents is to minimise matrix interference and increase sensitivity and life of instrument. The bottle guard fruit should be free from any pesticides befor going for extraction and analysis.

GC-FPD (GC-QP 2010 model) Shimadzu make with AOC-20S Auto Sampler was used for analysis. DB-5MS fused silica capillary column (Agilent J&W GC column, 5% Phenylated methyl siloxane), was used for separation of 23 pesticides which is of 30 m length × 0.25 mm i.d. × 0.25 µm film thickness was used for screening and quantification of pesticide residues. The oven programming was set at 100°C for 2 min with a ramp of 25°C/min up to 200°C for 5 min., then 4°C/min ramp upto 230°C for 2 min and 20°C/min to final temperature of 280°C with a hold time of 5 min. The injector port temperature was 250°C and detector temperature was, 290°C. Injection volume 1.0 micro litre and 0.5 min equilibrium time. The instrument works in split mode of (10:1). Helium gas was used as makeup gas and carrier gas at a flow rate of 1.23 mL/min. H_2 and air for combustion of flame with flow of 85 ml/min and 110.0 ml/min respectively.

Certified Reference Materials (CRM) of pesticide of above 95% was weighed in clean volumetric flask of of 10 ml. The standard solution of about 100 ppm was prepared using HPLC grade acetone and hexane solvent. The working standard was prepared by serial dilution from standard stock solution which should be kept at -20°C. The calibration standard solution was prepared at seven different concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00 mg/kg are considered for study. All working standard solutions of a mixture of pesticides were prepared for calibration and recovery tests.

Sample Preparation

The present environmental load of the pesticide residues is increasing day by day. It is important to determine the amount of pesticide residues in vegetable samples in and around Satna, Madhya Pradesh, India. Samples were prepared according to the QuEChERS (quick, easy, cheap, effective, rugged and safe) method [Anastassiades, M., *et al.,* (2003)] with some modifications. The estimation of commonly used pesticides for a period of one year. Spinach was chopped and homogenized, 15 gm of homogenized sample was taken in 50 ml centrifugation tube and added 30 ml of Ethyl acetate later shaken for 1 min then 10 gram anhydrous Na_2SO_4 was added and shaken for 1 min. 6 ml extract used for ceaning up according to Lehotay (2007). 0.9 g anhydrous $MgSO_4$, 0.25 g PSA and 0.25 g Activated charcoal was used for cleaning to remove matrix effect of highly pigmented foods. The supernatant 4 ml of cleanup extract was dried and 1 ml n hexane was added for injection in GCFPD and GC-MS.

Theory of uncertainty estimation

Uncertanity is very important steps of test results performed in any accredited laboratory. Following EURACHEM/CITAC guidelines, calculation of measurement uncertainty of 23 organophosphorus pesticides in bottle gourd occurs during standard solution preparation purity of standards, weight sample and certified reference standard , repeatability of results, recovery percentage, purity of CRM and Gas Chromatography responses.

Uncertainty involves measurand specificity, identity of uncertainty sources, quantification of uncertainty sources. The standard deviation value of sources which can be directly used, declared uncertain value in certificate and confidence level can be used for calculating uncertainty. Standard uncertainty (u(x)), is standard deviation of values, combined standard uncertainty is the sum of the square and their square root of all uncertainties whereas expanded uncertainty is (U(x)) is calculated from a combined standard uncertainty and a coverage factor k. Relative standard uncertainty (u rel) value is obtained as the division of standard uncertainty u(x) and the value of x. Formulas of the uncertainty mentioned are given below.

Standard Uncertanity u(x)= standard deviation values

urel(x) = u(x)/x

Combined Uncertanity (u) = square root of ((x_* x) + (y_* y)+…)

Expanded Uncertanity U(x)= (u)* coverage factor

Results and Discussion

For estimation of 23 organophosphorus pesticide in bottle guard. The study was conducted and method of analysis was validated. During validation step there are many sources of uncertainty arises. Steps involved are identification of uncertainty sources, quantification of uncertainty sources and overall calculation of the combined standard uncertainty. Uncertanity consists of random and systematic errors. All the errors are quantified and included in the combined standard uncertainty. As per the statistical procedure of the EURACHEM/CITAC Guide CG-4 [Eurachem/CITAC Guide, 2000]. Many sources of uncertainty arise in multiresidue method due to gravimetric and volumetric steps. Major sources of uncertainty due to purity of CRM, weighing, recovery and repeatability of results. Combined uncertainty (U) was calculated at 0.05 mg/kg level.

Pesticides taken for study have their specific purity percent. Standard uncertainty by purity of analytical standards (U1) was calculated from uncertainty value given in the certificate. Uncertainty value u(x) is divided by √3 (rectangular distribution) so the formula is-. U1 = (u (x) /√3). From uncertainty table 1, uncertainty of all pesticides CRM purity are almost same i.e 0.05 which is converted to (0.05/√3).

The uncertainty of the weighing (U2) is taken during weighing of 1–2 mg of Certified Reference

Table 1: Shows the uncertainty calculation due to purity of certified reference standards.

S. No.	Pesticide Standard	Purity of Standard	Wt. std	Uncertainity	Uncertanity of Standard SU1	Relative Standard Uncertanity (U1)
1	Dichlorvos	98.5	1.25	0.25	0.1443	0.1465
2	Monocrotophos	96	1.36	0.25	0.1443	0.1504
3	Phorate	99.4	1.54	0.25	0.1443	0.1452
4	Dimetoate	96	1.67	0.25	0.1443	0.1504
5	Diazinon	98.9	1.32	0.25	0.1443	0.1459
6	Paraxon-methyl	97.2	1.98	0.25	0.1443	0.1485
7	Phosphomidon	98.9	1.56	0.25	0.1443	0.1459
8	Fenthion	99	1.87	0.25	0.1443	0.1458
9	Chlorpyrifos-methyl	99.7	1.78	0.25	0.1443	0.1448
10	Parathion methyl	99.7	1.46	0.25	0.1443	0.1448
11	Fenitrothion	99.5	1.56	0.25	0.1443	0.1451
12	Malathion	99.5	1.35	0.25	0.1443	0.1451
13	Chlorpyrifos	99.3	1.67	0.25	0.1443	0.1454
14	Parathion	98.8	1.4	0.25	0.1443	0.1461
15	Chlorfenvinfos	99.5	1.98	0.25	0.1443	0.1451
16	Quinolphos	99.3	1.68	0.25	0.1443	0.1454
17	Fenamiphos	96	1.59	0.25	0.1443	0.1504
18	Profenofos	99.2	1.67	0.25	0.1443	0.1455
19	Ethion	98	1.37	0.25	0.1443	0.1473
20	Trizophos	97.8	1.49	0.25	0.1443	0.1476
21	Edfinphos	98.5	1.67	0.25	0.1443	0.1465
22	Anilophos	98.4	1.32	0.25	0.1443	0.1467
23	Phosalone	98.6	1.37	0.25	0.1443	0.1464

Material. Considering normal distribution of weight is taken in weighing balance, the uncertainty value i.e 0.05 gm. Standard uncertainty is the uncertainty of weighing balance divided by normal distribution (2) and relative standard uncertainty is standard uncertainty divided by the weight of pesticide standard weighted using precision analytical balance of 0.05 uncertainty value at 95% confidence level. The calculation of uncertainty value occurs due to weighing of CRM are calculated in Table 2.

Uncertainty arises due to precision (U3) of 23 organophosphorus pesticides. Standard deviation and relative standard deviation of repeatability were calculated. Repeatability was calculated by equation: $U3 = s/(\sqrt{n} \times x)$ where standard deviation (s) is obtained from the recovery study, n is the number of replications and x is the mean value of the concentration recovered (Table 3).

Table 2: Shows the uncertainty calculation due to weighing of certified reference standards.

S. No.	Pesticide Standard	Weight of Standard	Uncertainty in Weighing	Standard Uncertainty	Relative Standard Uncertainty (U2)
1	Dichlorvos	1.25	0.05	0.025	0.020
2	Monocrotophos	1.36	0.05	0.025	0.018
3	Phorate	1.54	0.05	0.025	0.016
4	Dimetoate	1.67	0.05	0.025	0.015
5	Diazinon	1.32	0.05	0.025	0.019
6	Paraxon-methyl	1.98	0.05	0.025	0.013
7	Phosphomidon	1.56	0.05	0.025	0.016
8	Fenthion	1.87	0.05	0.025	0.013
9	Chlorpyrifos-methyl	1.78	0.05	0.025	0.014
10	Parathion methyl	1.46	0.05	0.025	0.017
11	Fenitrothion	1.56	0.05	0.025	0.016
12	Malathion	1.35	0.05	0.025	0.019
13	Chlorpyrifos	1.67	0.05	0.025	0.015
14	Parathion	1.4	0.05	0.025	0.018
15	Chlorfenvinfos	1.98	0.05	0.025	0.013
16	Quinolphos	1.68	0.05	0.025	0.015
17	Fenamiphos	1.59	0.05	0.025	0.016
18	Profenofos	1.67	0.05	0.025	0.015
19	Ethion	1.37	0.05	0.025	0.018
20	Trizophos	1.49	0.05	0.025	0.017
21	Edfinphos	1.67	0.05	0.025	0.015
22	Anilophos	1.32	0.05	0.025	0.019
23	Phosalone	1.37	0.05	0.025	0.018

Table 3: Shows Recovery, Mean Recovery, Standard Deviation (S.D), and Relative Standard Deviation (RSD) of Organophosphorus pesticides from spiked bottle gourd matrix at 0.05 ppm.

S. No.	Pesticide	Spike Conc	Amount Recovered	Amount Recovered	Amount Recovered	Mean Recovery	S.D.	R.S.D
1	Dichlorvos	0.05	0.046	0.04	0.045	0.0437	0.0032	7.3626
2	Monocrotophos	0.05	0.044	0.048	0.042	0.0447	0.0031	6.8396
3	Phorate	0.05	0.04	0.038	0.04	0.0393	0.0012	2.9364
4	Dimetoate	0.05	0.043	0.045	0.042	0.0433	0.0015	3.5262
5	Diazinon	0.05	0.042	0.04	0.046	0.0427	0.0031	7.1602
6	Paraxon-methyl	0.05	0.043	0.043	0.042	0.0427	0.0006	1.3523
7	Phosphomidon	0.05	0.042	0.046	0.042	0.0433	0.0023	5.3285
8	Fenthion	0.05	0.043	0.041	0.046	0.0433	0.0025	5.8085
9	Chlorpyrifos-methyl	0.05	0.042	0.043	0.044	0.0430	0.0010	2.3256
10	Parathion-methyl	0.05	0.046	0.044	0.047	0.0457	0.0015	3.3460
11	Fenitrothion	0.05	0.044	0.043	0.04	0.0423	0.0021	4.9181

Total uncertainty is calculated by considering, relative uncertainty due to purity of standard (U1), due to weighing (U2) and precision (U3). For calculating combined uncertainty, the sum of the square root of U1, U2 and U3 are taken. The combined uncertainty (U) was calculated by equation: $U = x\ [(U1)^2+(U2)^2+(U3)^2]^{1/2}$. Expanded uncertainty (2U) was twice of combined uncertainty (U) at 95% confidence level (Table 4).

S. No.	Pesticide	Spike Conc	Amount Recovered	Amount Recovered	Amount Recovered	Mean Recovery	S.D.	R.S.D
12	Malathion	0.05	0.039	0.042	0.04	0.0403	0.0015	3.7884
13	Chlorpyrifos	0.05	0.043	0.046	0.039	0.0427	0.0035	8.2313
14	Parathion	0.05	0.046	0.043	0.04	0.0430	0.0030	6.9767
15	Chlorfenvin-fos	0.05	0.043	0.044	0.046	0.0443	0.0015	3.4466
16	Quinolphos	0.05	0.045	0.042	0.041	0.0427	0.0021	4.8797
17	Fenamiphos	0.05	0.045	0.043	0.046	0.0447	0.0015	3.4209
18	Profenofos	0.05	0.043	0.046	0.045	0.0447	0.0015	3.4209
19	Ethion	0.05	0.043	0.042	0.046	0.0437	0.0021	4.7679
20	Trizophos	0.05	0.046	0.044	0.046	0.0453	0.0012	2.5478
21	Edfinphos	0.05	0.043	0.045	0.048	0.0453	0.0025	5.5522
22	Anilophos	0.05	0.046	0.043	0.044	0.0443	0.0015	3.4466
23	Phosalone	0.05	0.046	0.043	0.047	0.0453	0.0021	4.5926

Table 4: Results of individual and combined uncertainties with expanded uncertainty for Organophosphorus pesticides from spiked bottle gourd matrix at 0.05 ppm.

S. No.	Pesticide	Purity %	Wt. std	Uncertainity	SU1	U1	U2	SD-Recovery	Mean-Recovery	U3	U	2U	% uncertainty
1	Dichlorvos	98.5	1.25	0.25	0.1443	0.1465	0.0200	0.0032	0.0437	0.000081	0.0065	0.0129	29.58
2	Monocrotophos	96	1.36	0.25	0.1443	0.1504	0.0184	0.0031	0.0447	0.000080	0.0068	0.0135	30.29
3	Phorate	99.4	1.54	0.25	0.1443	0.1452	0.0162	0.0012	0.0393	0.000027	0.0057	0.0115	29.22
4	Dimetoate	96	1.67	0.25	0.1443	0.1504	0.0150	0.0015	0.0433	0.000037	0.0065	0.0131	30.22
5	Diazinon	98.9	1.32	0.25	0.1443	0.1459	0.0189	0.0031	0.0427	0.000076	0.0063	0.0126	29.43
6	Paraxon-methyl	97.2	1.98	0.25	0.1443	0.1485	0.0126	0.0006	0.0427	0.000015	0.0064	0.0127	29.81
7	Phosphomidon	98.9	1.56	0.25	0.1443	0.1459	0.0160	0.0023	0.0433	0.000057	0.0064	0.0127	29.36
8	Fenthion	99	1.87	0.25	0.1443	0.1458	0.0134	0.0025	0.0433	0.000062	0.0063	0.0127	29.28
9	Chlorpyrifos-methyl	99.7	1.78	0.25	0.1443	0.1448	0.0140	0.001	0.043	0.000025	0.0063	0.0125	29.09
10	Parathion methyl	99.7	1.46	0.25	0.1443	0.1448	0.0171	0.0015	0.0457	0.000040	0.0067	0.0133	29.16
11	Fenitrothion	99.5	1.56	0.25	0.1443	0.1451	0.0160	0.0021	0.0423	0.000051	0.0062	0.0123	29.19
12	Malathion	99.5	1.35	0.25	0.1443	0.1451	0.0185	0.0015	0.0403	0.000035	0.0059	0.0118	29.25
13	Chlorpyrifos	99.3	1.67	0.25	0.1443	0.1454	0.0150	0.0035	0.0427	0.000086	0.0062	0.0125	29.22
14	Parathion	98.8	1.4	0.25	0.1443	0.1461	0.0179	0.003	0.043	0.000074	0.0063	0.0127	29.44
15	Chlorfenvinfos	99.5	1.98	0.25	0.1443	0.1451	0.0126	0.0015	0.0443	0.000038	0.0065	0.0129	29.12
16	Quinolphos	99.3	1.68	0.25	0.1443	0.1454	0.0149	0.0021	0.0427	0.000052	0.0062	0.0125	29.22
17	Fenamiphos	96	1.59	0.25	0.1443	0.1504	0.0157	0.0015	0.0447	0.000039	0.0068	0.0135	30.23
18	Profenofos	99.2	1.67	0.25	0.1443	0.1455	0.0150	0.0015	0.0447	0.000039	0.0065	0.0131	29.25
19	Ethion	98	1.37	0.25	0.1443	0.1473	0.0182	0.0021	0.0437	0.000053	0.0065	0.0130	29.68
20	Trizophos	97.8	1.49	0.25	0.1443	0.1476	0.0168	0.0012	0.0453	0.000031	0.0067	0.0135	29.71
21	Edfinphos	98.5	1.67	0.25	0.1443	0.1465	0.0150	0.0025	0.0453	0.000065	0.0067	0.0133	29.46
22	Anilophos	98.4	1.32	0.25	0.1443	0.1467	0.0189	0.0015	0.0443	0.000038	0.0066	0.0131	29.58
23	Phosalone	98.6	1.37	0.25	0.1443	0.1464	0.0182	0.0021	0.0453	0.000055	0.0067	0.0134	29.50

SU1 = Standard uncertainity of analytical standards

U1 = Relative Standard Uncertainity of analytical standards

U2 = Relative Standard Uncertainity of weighing

U3 = Uncertainity associated with precision

U = Combined Uncertainity

2U = Expanded Uncertainity

Conclusion

Twenty-three organophosphorus pesticides uncertanity estimation of in bottle gourd matrix shows that the values obtained are within permissible limit as per codex and EURACHEM/ CITAC (2000) guidelines. Major sources of uncertainty common to most all methods are considered. As the experiment is performed in well equipped, NABL, and BIS accrediated lab, the data generated is realistic and trustful. So the data can be used for reporting of results and also helps laboratory in extension of analysis of scope.

Aknowlegement

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Declaration

Authors made declaration that the manuscript is our own original work and it does not duplicate any other previously published work. Authors do not have any conflict of interest.

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Review Article

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Forensic Investigation of Explosions: A Review

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Abstract

An explosion is defined as a violent, shattering action caused by a bomb. The main chemical ingredient of a bomb is the explosive – an endothermic substance which serves as a storehouse of energy. When this energy is suddenly released, in the confined space of the bomb, it causes a devastating effect, resulting in loss of lives and property. As compared to conventional crime scenes, explosion sites are more difficult to process. In many cases a building may have collapsed and the crime scene evidence may have become buried beneath the debris. A vital aspect of forensic investigation of bomb blasts is to establish the explosion seat. Equally important is to identify the type of chemical explosives used to commit the crime. This communication highlights the complications encountered in the management of explosion sites and the difficulties experienced in processing the evidence collected there from. The types of injuries which the victims of explosion suffer are also briefly described.

Keywords: Blast waves; Blasting caps; Booster; Deflagration; Detonation; Explosive; Explosive charge; Explosive train; Initiator.

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Introduction

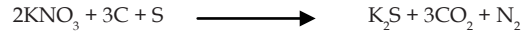
An explosive is a substance or a mixture of substances which, when raised above a threshold temperature, whether by direct heating, friction, impact or shock from another explosive, undergoes a violent and spontaneous chemical transformation with the evolution of a large amount of heat, light and sound, as well as with release of a large volume of gases.[1] The gases, so released, exert a high pressure on the surrounding media.[2] The amount of material required to cause an explosion is called *explosive charge*. The explosive charge stores a great amount of potential energy which, at the moment of explosion, is released as chemical energy, a part of which dissipates as heat, light and sound.[3]

An eruption of explosive has two similarities with an incidence of fire. Firstly, both are combustion reactions. Secondly, both require a fuel and an oxidizing agent. However, whereas in fire, the combustion reaction proceeds smoothly, at a slow rate, in an explosion it occurs at a phenomenal rate, causing violent physical disruption of the surrounding area. Moreover, in arson, aerial oxygen acts as the oxidizing agent, but in explosives, an oxygen-rich functional group is the oxidant.[4]

Mechanism of Explosions

Depending on the pathway of the explosion reaction, the explosives are categorized into

two types: composite explosives and molecular explosives.[5] A *composite explosive* is one in which the fuel and the oxidizing agent are separate chemical entities. *Gun powder*, also called *black powder* is an example of a composite explosive.[6] It is a mixture of potassium nitrate (75%), charcoal (15%) and sulphur (10%). Potassium nitrate acts as an oxidant, charcoal as fuel and sulphur lowers the threshold of ignition and is thus a sensitizer. When gun powder is heated, the following chemical reaction takes place.



$$2KNO_3 + 3C + S \longrightarrow K_2S + 3CO_2 + N_2$$

The high pressure produced due to the release of large volumes of carbon dioxide and nitrogen causes the explosion. Another example of a composite explosive is ammonium nitrate – fuel oil, commonly abbreviated as ANFO. In this formulation, ammonium nitrate is the oxidant for fuel oil.[7]

It is also possible that the oxidant and the fuel are a part of the same molecule. Such an explosive is called a *molecular explosive*. Examples of molecular explosives include nitroglycerine, trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), nitrocellulose and cyclorite (RDX) [8]. Their structures are depicted in Fig. 1. In these compounds, the functional groups act as oxidants, while the carbon chain acts as the fuel.

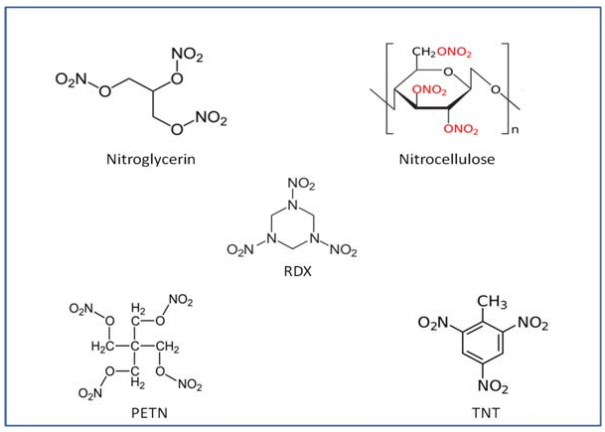
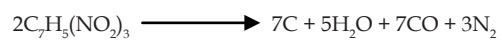


Fig. 1: Common molecular explosives

For example, the explosion reaction of trinitrotoluene may be written as follows.



$$2C_7H_5(NO_2)_3 \longrightarrow 7C + 5H_2O + 7CO + 3N_2$$

Since carbon is one of the products of the reaction, a black cloud appears at the site of TNT explosion.[5]

Classification of Explosives

Explosives are broadly classified into two types: low explosives and high explosives (Fig. 2).[9] Low explosives are those which decompose with a rate lower than the velocity of sound. It is generally within the range 0.20-300 ms^{-1}. As a result, their eruption is not very violent and is termed as *deflagration*. The latter does not initiate from within the main body of charge, but proceeds from the surface, consuming the inner layers step by step. The action is therefore only mildly shattering. Low explosives are also called propellants, implying that these are mainly used to push the ammunition out of the firearm. Gun powder, which is commonly used to propel the bullets from a rifle, is an example of a low explosive. Smokeless powder, the main ingredient of which is nitrocellulose, is yet another example of a low explosive.

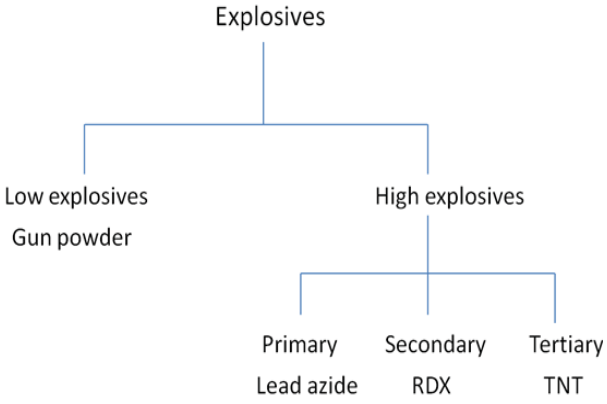


Fig. 2: Classification of explosives

High explosives are those whose rate of decomposition is of the order of 1000–9000 ms^{-1}. The explosive action originates from within the main body of charge and results in a powerful mechanical effect called *detonation*.[4,9] High explosives are sub-classified into three types: primary, secondary and tertiary.[1]

A *primary high explosive* is one which is extremely sensitive to shock, friction or mild heat.[11] Examples include lead azide and mercury(II) fulminate. Such explosives are difficult to handle and transport. These are mainly used to initiate the detonation of a secondary high explosive and hence are also referred to as *initiators* or *detonators*.[4]

Secondary high explosives are insensitive to impact or heat. These are quite stable and can be handled and transported relatively easily. Only when subjected to shock waves, do these explode.

The shock wave is provided by a primary high explosive (Fig. 3). Thus lead azide is a commonly used detonator for secondary explosives like PETN or RDX.[1]

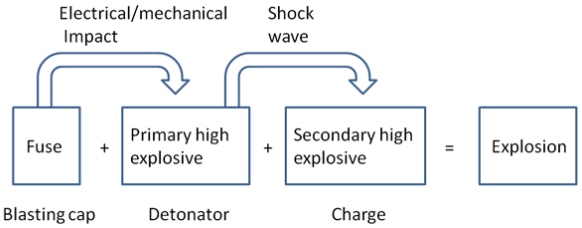


Fig. 3: Eruption of a secondary high explosive

In order to enhance the shattering effect, the primary and secondary high explosives are packed together in tubes made of copper or aluminium. Such tubes are called *blasting caps*.[1] These are usually 5 mm in diameter and 25-75 mm in length. One variety is shown in Figure 4.

Fig. 4: A blasting cap

The blasting caps are endowed with a fuse which operates either electrically or mechanically to explode the primary high explosive, generating the shock wave to erupt the secondary high explosive.

A *tertiary high explosive* has an enormously high shattering power but, paradoxically, a high stability as well.[3] Once a tertiary high explosive erupts, the devastation will be phenomenal, but for initiating its explosion, a detonator in form of a primary high explosive is not enough. Rather a combination of a primary high explosive and a secondary high explosive is needed to blast a tertiary high explosive.

The primary high explosive first detonates the secondary high explosive and the high-order shock wave, so generated, triggers the eruption of tertiary high explosive. The intermediate, secondary high explosive, which amplifies the shock wave of the primary high explosive is called *booster*.[5] For example, RDX is a booster for the teritiary high explosive like nitroglycerine. The sequence of explosion of a tertiary high explosive is symbolically shown in Figure 5.

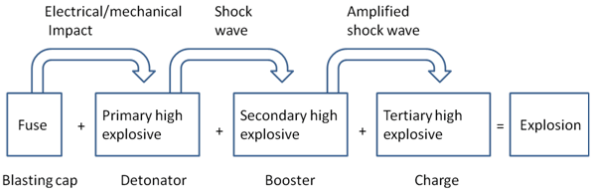


Fig. 5: Eruption of a tertiary high explosive

The series of operations, initiating with the fuse and culminating in an explosion, as shown in Figs. 3 and 5, are called *explosive trains*.[12]

Management of Explosion Site

The objectives of explosion scene management are twofold: firstly to collect evidence and secondly to redeem the area or the infrastructure at the earliest. Before resorting to bomb scene management, emergency measures such as providing medical aid to the injured and extinguishing fire should be pressed into service. Whereas in a conventional crime scene, a single cordon is sufficient, a bomb site ought to be secured by two barrier tapes. The inner cordon covers the area immediately surrounding the bomb seat, while the outer cordon is deployed a few meters away. The area enclosed by the inner cordon is restricted to experts in the field of explosives and evidence collection. The space between the inner and outer cordons is used for keeping technical and documenting tools at hand should these be requisitioned by the experts.[13]

Those entering the inner area ought to wear protective clothing, helmets, gloves and shoe covers. They should also wear masks to avoid inhaling toxic fumes and fine dust particles. The road traffic immediately beyond the outer cordon should be diverted. A metro station, if in vicinity, should be closed. An area for press briefing should be designated outside the second cordon.[13,14]

The area within the inner cordon is the one where most of the crime scene evidence would be found. The first order of business for the investigators is to conduct a walk through of this area so as to acquire an overview of the incident. During this exercise, they should establish the entry and exit pathways for the experts who would subsequently join in the investigation. They should also divide the area within the inner cordon into a suitable number of zones. Each zone is then methodically scanned for plausible evidence. Once the first zone has been thoroughly probed, investigators should move to the second zone, and so on.

The zone where the bomb actually explodes warrants the most painstaking search.[15] At the bomb

seat a crater is invariably formed. The dimensions of the crater are recorded and thereafter it is further excavated to a depth of about 0.3 meter so as to extract the evidence buried therein. All loose soil and debris is removed from the interior of the crater and screened through wire mesh to sift out the paraphernalia used in detonation, such as wires fuses, nails and shells. The soil too is preserved for extraction and subsequent analysis of explosive residues which may have become mixed up with debris. Explosive residues are also commonly found adsorbed on items like door panels, window sills and glass pieces that were shattered due to the bomb blast. Volatile residues may be found absorbed in porous items like wood, carpets, curtains and rags. A set of evidence may be blown away from the bomb seat under the impact of blast waves. These will be found in zones away from the crater.

All pieces of evidence should be picked up by disposable scoops, scrapers, dustpans and brushes. If vacuum collection is warranted, the vacuum cleaner should be endowed with disposable accessories. Cotton swabs may also be used as picking aids either in dry state or after moistening with a suitable solvent.

All the collected items should be packed in air tight metal containers. Soil and debris collected from different zones should be packed in separate containers. The type of evidence and the zone number from which it was picked up should be clearly marked on the container.[16] Plastic bags should not be used for packaging explosion evidence since the residues interact with and degrade the polymeric materials. Moreover, sharp edged objects like wires and nails may pierce through the plastic bags and fall off. Paper envelops too are unsuitable since the volatile explosive residues are likely to ooze out of the container.

The investigators should prepare written scene documentation for permanent record. The date on which the explosion occurred, the time at which the information received and the time of arrival at the scene should be indicated in the report. The location, physical measurements and environmental conditions too should be stated. The interviews of witnesses should be incorporated in the report. A rough sketch of the scene, clearly marking the crater, visible evidence and dead bodies should be drawn at the site itself. It should be subsequently fine-tuned either manually or better by making use of computer aided drawing software. The overview of the scene should be photographed, followed by photographing each zone separately. The bomb seat should be photographed from all possible angles. A mid-range photo log showing relative location of two or more evidence should be prepared. In addition, a close-up photograph of each evidence should be taken. Photography and videography of the assembled crowd should also be carried out. This assists in identifying witnesses, and in some cases, the suspects as well.[15,17]

Analysis of Explosion Evidence

Every item from the bomb blast site is first examined with the aid of a microscope to detect particles of unconsumed explosive.[12] These are more likely to be found adhering to the detonator parts than in the embedded debris. If ammonium nitrate is suspected to be the explosive, the evidence is rinsed with water. For other explosives, the evidence is rinsed with acetone. A preliminary examination may be performed by carrying out spot tests with diphenylamine and Griess reagents on the water or acetone extracts.[18] As shown in Table 1, these reagents give characteristic colored spots with all common explosives except trinitrotoluene. The latter, nevertheless, gives a red spot when treated with alcoholic potassium hydroxide.[1,12]

Table 1: Spot tests for common explosives

Explosive	Griess reagent	Diphenylamine reagent	Alcoholic KOH
Nitrate	Pink	Blue	No color
PETN	Pink	Blue	No color
RDX	Pink	Blue	No color
Nitroglycerine	Pink	Blue	No color
TNT	No color	No color	Red

For final confirmation, the washings from water or acetone are concentrated and analyzed with the aid of thin layer chromatography or high performance liquid chromatography.[19,20] Gas chromatography is not routinely used since it operates at high temperatures – and this may prove unsafe while analyzing explosives.

Another device which separates the compounds present in an explosive residue is the ion mobility spectrometer.[1] This hand-held instrument uses vacuum to collect explosive residues from suspect surfaces. Once inside the ion mobility spectrometer, the residues are vaporized by using a heat source. The vapors are exposed to β-rays (electron beam) emitted by radioactive nickel. The chemical entities are converted into electrically charged particles which are then allowed to move in a tube under

the influence of an electric field. The preliminary identification of the residue components can be made by noting the time taken by these to move through the tube. The speed depends on their size and structure. The segregated components are finally analyzed by a chromatographic technique.[21]

Blast Waves

A blast wave refers to the flow of hot, compressed gases liberated as fallout of an explosion. The gases are pushed by the large amount of energy released in a limited space of a bomb shell. The wave expands rapidly as it moves away from the bomb seat and, in consequence, compresses the surrounding air. In fact, blast wave is a propagating disturbance that shatters anything that gets in its way. Debris, common utilities, components of detonator and sometimes even people get swept away by it. As the wave moves forward with supersonic speed, it creates a partial vacuum immediately behind it due to displacement of air. To fill up the vacuum, the hot gases of the wave take a U-turn and rush back towards the bomb seat. This causes another blast effect called *negative pressure phase*. The latter is not as devastating as the positive blast phase, but is capable of causing additional damage to the objects that have already been damaged. It may also trigger additional injuries to the victims who survived the direct blast waves.[22] The physical trauma suffered by the victims who come in the line of blast waves are classified into four types: primary, secondary, tertiary and quaternary.[23]

Primary blast injuries are caused to a person who happens to be close to the bomb seat at the time of explosion. The trauma results due to combined effect of pressure and shock embodied in the blast wave and affects internal organs like ear drums, lungs and gastrointestinal tract.

Secondary blast injuries are caused by components of the detonator, such as nails and glass fragments which are propelled outwards during the explosion. These may cause both internal and external injuries to a person who need not only be close to the bomb seat, but may be at a remote location.

Tertiary blast injuries appear in form of bone fractures, blunt trauma and tissue rupture to a person who is lifted by the blast wave to strike a hard object. Children are more prone to such injuries.

Quaternary blast injuries encompass disorders not covered under the aforementioned three classifications. These include burns, amputation of limbs, blinding and nerve damage. Post-trauma psychic disorders may also appear in those persons who may have escaped physical injuries but had witnessed the scene of disaster.[24]

Conclusion

The forensic investigation of explosions entails the following steps.

- Identification of the bomb seat
- Collection and analysis of explosive residues
- Identification of explosive or mixture of explosives used to commit the crime.
- Examining the damage caused by blast waves.

The complexities and difficulties associated with processing of explosion scenes may be negated if the aforementioned steps are carried out scientifically and methodically.

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Case Series

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Utilizing Toxidromal Approach in Managing Series of Botanically Related Medicolegal Emergencies

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Abstract

As with many other medicolegal dilemma in poisoning, in which whom to blame for the toxic exposure, and who should be punished. Since after the decriminalization of suicidal attempt under section 309 IPC, victim himself can't be held responsible for the harm caused to him/her by self. But abatement of suicide is still a crime, so if someone harasses another person physically or mentally or psychologically, and the victim consumes some toxic plant product under stress, than it becomes a medicolegal case to be reported to police, to protect the victim and restrain the accused for causing further harm. Bedside care of patients with toxic plant exposures should be managed primarily based on their clinical manifestations and responses to therapy and only secondarily on the basis of the toxin to which they are presumably exposed. The dictum has been and remains "Treat the patient, not the poison". But don't ignore the poison. We report few interesting cases reported in our ER: self-intoxication during voluntary ingestion of nutmeg-myristicinas aphrodisiac, self-overdose of Calotropis seeds as purgative by young female, Homicidal Atropa seeds toxicity in a child, Areca Nut aspiration by male Gutkha chewer, Opium intoxication in youth, Oriental Starfruit causing nephrotoxicity, Bottle gourd juice causing UGI Bleed, Cannabis abuse simulating Acute coronary syndrome. Toxidromal Approach simplified theapproach to differential diagnosis and emergency management of symptomatology and aetiology of intoxication, thus saving lives and improving quality of acute care.

Keywords: Toxidrome; Atropa; Nutmeg; myristicin; Calotropis; Intoxication; Overdose; Starfruit; Bottle gourd; Areca Nut; Cannabis; Opium; Aphrodisiac.

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Background

We studied botany before, and then we all studied medicine including Toxicology. Herbal remedies, on psychoactive and toxic plants, especially of the tropics. Initially, through stories of the indigenous lifestyle of Village peoples, and later by helping me undertake fieldwork in this region, it awoke in me a keen interest in the botany of useful plants that led me to become first an investigator and later a practitioner of botanical medicine. When I moved on to Medical College, We weredismayed to find that none of my teachers, even of pharmacology, had firsthand knowledge of the plant sources of drugs. Since then we have been continually struck by the lack of awareness of the medicinal and toxic properties of plants in our culture. Examples are unfounded fears of poisoning by common ornamentals such as the poinsettia, exaggerated

fears of herbal remedies such as Chinese ephedra, ignorance of the vast medicinal importance of such spices as turmeric and ginger, and lack of awareness of the toxic and psychoactive properties of other spices, for example, nutmeg and mace. Specific identification of a plant may guide management by revealing potential toxins, placing the risk in context, and providing a time frame for the development of clinical findings.

Care should be taken to avoid misidentification, a particular problem when plants are discussed by their common rather than by their botanical name. Although management of a patient with an identified exposure is generally preferable to managing a patient with an "exposure to an unknown plant," many plant-exposed cases are managed successfully without knowledge of the culprit plant. However, adverse events may result by the attempted management of a misidentified plant. Perhaps the greatest paradigm shift in recent years is thecurrent de-emphasis of aggressive gastrointestinal decontamination. Syrup of ipecac, for example, is almost never recommended, and orogastric lavage should be reserved for those patients with a reasonable likelihood of developing consequential poisoning.

This group should include the minority of patients exposed to plants. Although oral activated charcoal is effective at reducing the absorption of many chemicals, its benefit following the vast majority of plant exposures has never been specifically studied. However, given the extremely low risk of administration of oral activated charcoal to an awake patient who is able to drink spontaneously, its use should be considered in patients with plant exposures. ISTOLS (Indian Society of Toxicology Life Support Course) training include descriptions of the clinical findings and focused descriptions of management strategies for patients with plant poisonings via Toxidromal approach.

Although very few antidotes are available to treat the effects of the innumerable toxins available in plants, rarely are antidotes actually necessary. Much of our understanding of poisoning syndromes derives from toxicity associated with theuse of purified plant toxins as pharmaceuticals (e.g., morphine from Papaver somniferum).

The amount of a toxin present in a plant is unpredictable, whereas the amount in a tablet is always defined. There is generally a lower concentration of "toxin" in the plant than there is of

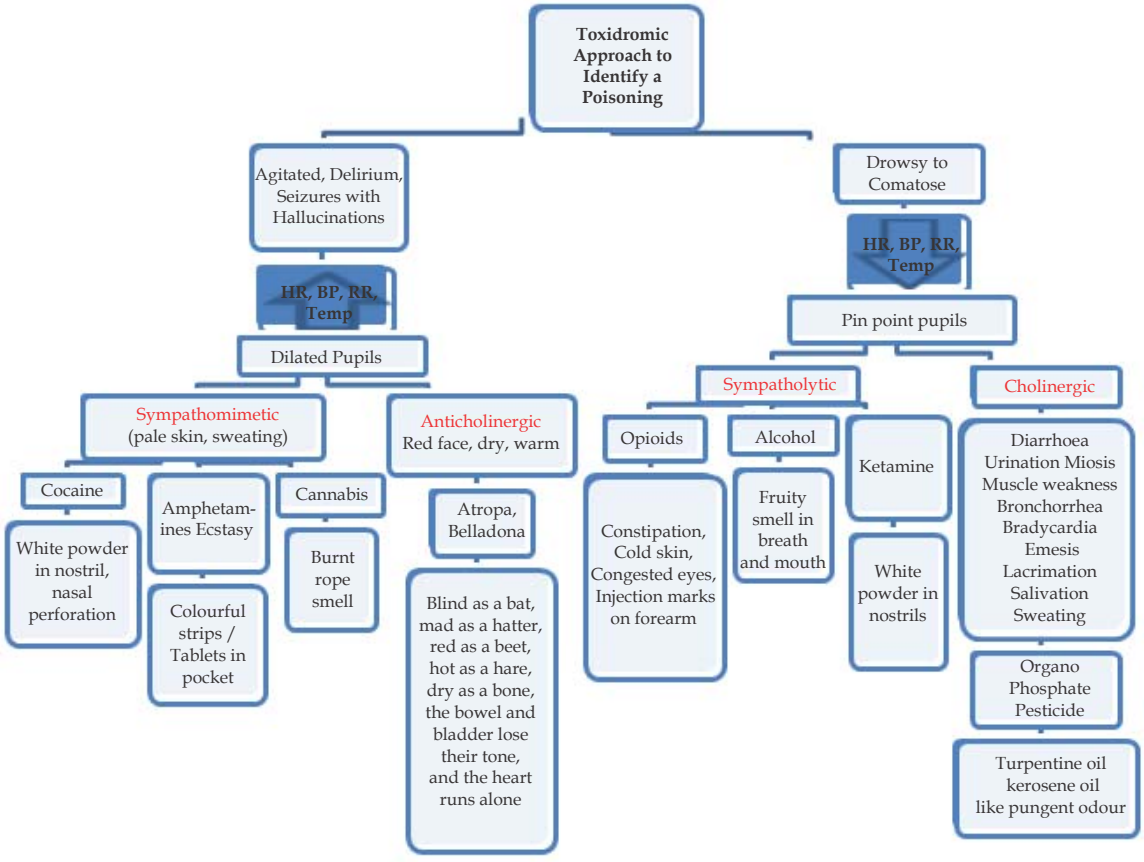

Fig. 1: Toxidromic Approach to Identify a Poisoning

"drug" in a tablet. However, this byno means should minimize the clinical concern following exposure to a plant containing a consequential toxin, such as Colchicum autumnale, which contains colchicine. The cost and effort associated with proving an exposure (e.g., toxin levels in blood) makes this task (unfortunately but appropriately) of low priority to the physician involved with the care of the exposed patient. As with many other clinical situations, bedside care of patients withtoxic plant exposures should be managed primarily based on their clinical manifestations and responses to therapy and only secondarily on the basis of thetoxin to which they are presumably exposed. The dictum has been and remains"Treat the patient, not the poison". But don't ignore the poison.

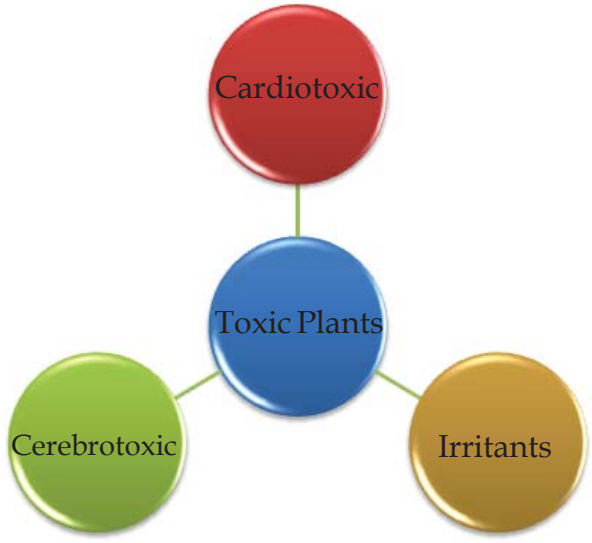

Fig. 2: Types of Toxic plants

Introduction

Poisonous Plants, Poisoning Syndromes, and Their Clinical Management:

General initial medical management strategies that are required for allplant-exposed patients include, but are not necessarily limited to, vital signassessment, consideration of the need for immediate interventions (e.g., ventilation and oxygenation, blood glucose), determination of the need for laboratory or other diagnostic testing, and the consideration of the need forgastrointestinal decontamination. Intervention at any point thatis deemed appropriate to correct or prevent progression of a clinical abnormalityis critical.

Toxidromic approach to management of plant poisoning:

Common poisonings may present with one or more of the following in the form of common Toxidromes, based on important diagnostic variables in the physical examination – sensorium and mental status, blood pressure, pulse rate, respiratory rate, oxygen saturation, body temperature, pupil size, profuse perspiration/ dry skin.

Types of Toxidrome

↑ Sympathomimetic ↑ Cholinergic - Parasympthomimetic

↓ Sympatholytic ↓ Anticholinergic- Parasympatholytic

Sympathomimetics act as antidote to sympatholytic overdose, and vice versa.

Cholinergics act as antidote to anticholinergic overdose, and vice versa.

Autonomic Nervous System (ANS) sustains the automatic functions of vital organs. Most of the fatal toxicants affect the ANS in one way or another, thus compromising the vital organs function, resulting in immediate death, if the toxicant was given in fatal dose.

Search for scene safety as per World Health Organization (WHO) protocol:

"Dr. ABCDE" Mnemonic. This follows the general principles of life support given below:

- Dr - ABCDE
- D - DANGER - scene safety
- R - RESPONSE- call for help
- A – AIRWAY secured and maintain patency/ Antidote
- B – BREATHING support
- C – CIRCULATION maintain access
- D - DISABILITY/ Decontamination - Whole body
- E – EXPOSE THE PATIENT completely - to find poisonous bites/stings
- F- Foley's catheterization for rapid excretion of toxic metabolites
- G-Gastric lavage for decontamination
- I- Isolation of patient & I-Intermittent rotation of Health Care provider to prevent further spread of toxicity to others (occupational hazards)

Anticholinergic Toxidrome

Tachycardia with mild hypertension is common,

and the body temperature is often elevated. Pupils are widely dilated. The skin is flushed, hot, and dry. Peristalsis is decreased, and urinary retention is common. Patients may have myoclonic jerking or choreoathetoid movements. Agitated delirium is frequently seen, and severe hyperthermia may occur. Examples: Atropa Belladona, Atropine, scopolamine, other naturally occurring anticholinergics, poisonous mushrooms. Deadly nightshade (Atropa belladona), Bittersweet (Solanumdulcamara), Black henbane (Hyoscyamusniger), Jimson weed (Dhatura species), Paneolinafoenisecii, Jerusalem cherry (Solanumpseudocapsicum), Potato (Solanumtuberosum).

Case Reports

Recent medicolegal Cases reported due to Poisonous Plants

Case Report 1: A previously well 28 year old male presented with complaints of palpitations, drowsiness, nausea, dizziness, thirst, and dry mouth. He was very anxious, restless, and agitated and described being "in a trance state". He specifically felt "like Jack in the box wanting to get out" but did not have hallucinations. He did not complain of urinary or abdominal discomfort and gave no history of seizures or migraine. He had an unremarkable medical and psychiatric history and denied any suicidal ideation. The patient on detailed historygave information regarding recreational drug use for prolonged sexual intercourse. This occurred with a newly-wed couple in an attempt for a low cost alternative to recreational drug use.

On examination the patient was agitated but alert, flushed but pyrexial, with a respiratory rate of 20/minute and saturation of 96% on room air. He was tachycardic at 102/minute with a blood pressure of 105/68. Cardiopulmonary examination was unremarkable. The abdomen was soft and non-tender. Cranial nerves were normal, while peripheral nervous system examination showed brisk, symmetrical deep tendon reflexes. There was no neck stiffness. Pupils were dilated to size 4 mm and were symmetrically brisk to light and accommodation.

A 12 lead ECG showed a fast sinus arrhythmia (rate 95–110/minute) with no ischaemic or hypertrophic changes. Serum urea, electrolytes, liver transaminases, full blood count, and serum catecholamines levels were normal. Serum and urine toxicology screens were negative.

In view of the complexity of his condition he was admitted in local hospital and five hours later referred to Medanta-the Medicity Hospital for having taken a large dose of nutmeg while trying to "get high". Some 30g of commercially available grated nutmeg were blended into a milkshake, the patient drankwhole of the amount. A feeling of elation was experienced in our patient, this was followed by his presenting symptoms 30 minutes after ingestion. The patient was kept for observation, offered reassurance, and rehydration. After symptoms had resolved he was allowed to return home 10 hours after presentation, 30 hours after ingestion.

Poisoning by Plants with Anticholinergic (Antimuscarinic) Poisons: Nutmeg poisoning is rare but probably underreported and should be considered in recreational substance users with acute psychotic symptoms as well as central nervous system neuromodulatory signs that may mimic in part an anticholinergic hyperstimulation.

Case Report 2: 12 yrs old male kid brought by his parents from nearby village for sudden onset abnormal behaviour, abusive, thus was harming self and others. On Examination, patient's pupils were dilated and reactive, and was restless, anxious, unable to sit, kicking the staff and doctors. Based on the typical toxidromal signs & symptoms of stimulant toxidrome of either anticholinergic intoxication or sympathomimetic toxidrome. Patient was calmed down with sympatholytic agents Benzodiapines and physically restrained, and we asked for history from the parents of the victim for any drug abuse or accidental exposure to any plant seeds, or any past history of similar episode, but nothing significant was found. MRI & EEG Brain were normal. We conducted urine for toxscreen, which revealed nil. Police intimation was done, who enquired all the family members, and patient's maternal grandfather confessed that he had deliberately intoxicated the patient with Atropa belladonna seeds containing atropine, to take revenge from his son-in- law (patient's father), for some property dispute in the village. Atropine neurotoxicity explained the typical toxidromal signs and symptoms of anticholinergic intoxication.

Sympathomimetic Toxidrome

The blood pressure and pulse rate are elevated, though with severe hypertension, reflex bradycardia may occur. The temperature is often elevated, pupils are dilated, and the skin is sweaty, though mucous membranes are dry. Patients are usually agitated, anxious, or frankly psychotic.

Examples: Magic mushrooms – Psilocybin, Ephedra plant-ephedrine and pseudoephedrine, Marijuana having Cannabinoids, Nux vomica seeds – Strychnine, Khat plant (Catha edulis) overdose.

Opioid Withdrawal - Sympathetic nervous system over-activity

Case Report 3: Interesting case of Medical Emergency associated with Chronic Cannabis toxicity. 24-year-old medical intern, who was discovered in a deeply comatose non-reactive state by his roommates in Boy's Medical college Hostel. Patient was shifted in ambulance and had 3 episodes of abnormal tonic clonic movements with frothing of mouth and received antiepileptic on way. According to the patient's roommates and attendants, patient had been a regular Marijuana smoker (up to 5-6 Marijuana cigarettes a day) for one year, and had increased smoking more than 15–16 Marijuana loaded cigarettes since last few days. Differential diagnosis:

- S-Stroke, or
- S-Seizure, or
- S-Sinus Thrombosis, or
- S-Sympathomimetic toxidrome.

By perusal of clinical findings and investigations by Toxidromal approach, We concluded that the patient had developed Cerbral Venous Thrombosis (CVT) with hemorrhagic cerebral infarcts due to chronic cannabis toxidrome, causing seizure (Fig. 3). Cannabis was the incriminated element in the cause of the thrombosis of our patient. Detailed workup of CVT was done which showed:-ANA-Antinuclear Antibody negative, Anti Cardiolipin antibody negative, IgG & Ig M Beta 2 Glycoprotein negative, Protein C and S Normal, Homocysteine within normal limits.

MRI scan of his brain revealed left sigmoid sinus thrombosis with acute venous hemorrhagic infarct involving left temporal lobe, posteroinferiorly

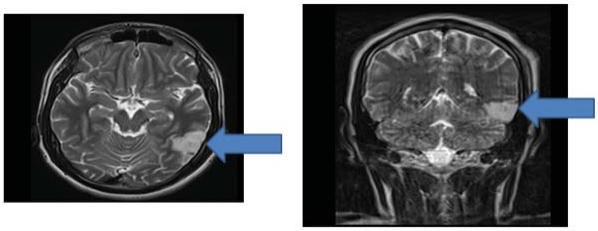

Fig. 3: Cannabis Toxicity

Discussion

Neurotoxic poisoning in the presence of associated injuries is commonly seen associated with trauma, and can result in devastating outcomes, if left unrecognized. Adequate information by doctors, who are mostly the first independent witnesses in poisoned victims, helps the police and court in investigation the suspected cases of trauma or toxicology, which can help in getting timely justice to the victims, and punishment to the culprits. As most exposures result in little or no toxicity, the initial management of most incidents involving patients who are asymptomatic should be expectant. This approach includes observation, at home or in the hospital as appropriate, depending on the nature of the exposure, and supportive care. For example, patients with several episodes of vomiting may benefit from an antiemetic agentand oral rehydration or, occasionally, intravenous fluids. At the root of this problem is the distance that exists between plant scientists and health scientists. Because I am trained in both worlds, I have been very conscious of it all my professional life. This intellectual gap creates difficulties for botanists who want to learn the medical significance of plants with pharmacological effects and for physicians, nurses, and pharmacists who want to learn how plants influence health, whether for good or ill. By bringing together specialists from both sides of this divide, the present article does a great service. It gives different perspectives on poisonous and injurious plants while remaining grounded in the integrative science of modern ethnobotany. Because of our background in botany, was often asked questions about the harmful potentials of plants and products derived from them. We meet many people who imagine that most wild plants are dangerous, who think that if you pick and eat plants at random in the backyard or woods you will die. In fact, the percentage of plants that are really harmful is quite small, as is the percentage that are really beneficial. If you wish to get toknow plants, a good place to start is to learn about those that can kill or cause serious harm.

Conclusion

The easy availability of environmental poisons like poisonous plants and their products commercially determines the choice of their use in society. Medicolegal Importance of neurotoxic plant poisons in homicidal, suicidal and accidental exposure is that they are fatal and hazardous to vulnerable population of society- especially kids, youth and young persons just after marriage. The effects are often fatal immediately after exposure and deprive them of those whom they know and

love. We described few cases of plant poisoning reported in Medanta Hospital Emergency, to understand the important role of doctors in giving logic and reasoning as expert opinion expressed for administration of justice and utilizing the Toxidromal approach of ISTOLS in better diagnosis and management, in the best interests of the patient.

Author's Contributions: All authors have read, reviewed and contributed to the final manuscript.

Conflict of Interest: Nil.

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Corporate (collective) author

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Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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