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Clinicohaemato-biochemical alterations in rats exposed to Datura stramonium seed toxicity

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Abstract
For this study, 63 rats of either sex, aged 4 weeks were randomly divided into three groups comprising 21 animals in each. The animals of group I provided distilled water and served as control, group II and group III were provided *Datura stramonium* seed extract @ 1000 and @ 2000 mg/ kg body weight orally, respectively for 90 days.

All the rats of both groups showed clinical symptoms of depression, reduced appetite, and dilatation of pupils (mydriasis), increased thirst, restlessness and excitation for a longer period. The values of Hb and PCV significantly decreased on day 30, 60 and 90 in group-III and on day 90 in group-II. TEC were significantly decreased at all intervals in group-III and 60th and 90th day in group-II. The TLC values showed highly significant leucopenia on 30, 60 and 90 day post feeding in rats of group-III and 60th and 90th days in group-II. There was highly significant neutrophilia at 30th, 60th and 90th day in the rats of group-III and on 60th and 90th day of the experimentation period in the rats of group-II. There was highly significant lymphocytopenia at 30th, 60th and 90th days of experimentation in the rats of group-III and on 90th day of experimentation in the rats of group-II. The value of AST, bilirubin, urea, cholesterol and catalase significantly increased in rats of group-II on day 90 and in the rats of group-III on day 60 and 90. The plasma uric acid revealed highly significant increase in the mean value on day 90 in rats of group-II and III. The value of plasma creatinine and alkaline phosphatase was highly significant increase on day 60 and 90 in group-II and on day 30, 60 and 90 in the rats of group-III. The mean value of total proteins was highly significant reduced in the rats of group-III on day 60 and 90. However, the mean value of albumin was highly significant decrease on day 60 and 90 in group-II and on day 30, 60 and 90 in the rats of group-III.

Keywords: Biology, brinjal, leucinodes orbonalis, morphometry

Introduction
Datura is one of the most investigated model plant containing tropane alkaloids. Various species of the genus *Datura* are found in barren land, cultivated land, and road sides. *Datura stramonium* is indigenous to India. The seeds have the highest alkaloid content compared to the flowers, stem, immature fruits and leaves [1]. Tropane alkaloids, namely Scopolamine, atropine and hyoscyamine (an isomer of atropine) are available as the main constituents of different parts of the plants [2]. Tropane alkaloids are readily absorbed following oral ingestion. Atropine is a central nervous system stimulant [3]. *Datura* plants are toxic for animals if ingested in larger amount. Grazing animals are unlikely to consume fresh *Datura* plants voluntarily because the plant has a very unpleasant taste and odour. Poisoning can accidently occur if *Datura* is present in hay or its seeds contaminate grains or oilseeds product fed to livestock, as animals do not detect these impurities in dried forms [4]. Poisoning appears to be an uncommon cause of illness in animals compared to other clinical problems such as infectious diseases, trauma or neoplasia. The effect of atropine on the heart rate, which commences as bradycardia at low (therapeutic) doses, progressing into tachycardia and arrhythmia at higher (toxic) doses [5].

There appears paucity of published reports of systematic studies done on *Datura* poisoning in animals. Keeping the above facts in view, this investigation has been designed to study the clinico-haematobiochemical changes of *Datura stramonium* seed extract toxicity in Wistar rats.

Materials and Methods
For this study, 63 rats of either sex, aged 4 weeks, weighing 100-120gms were randomly divided into three equal groups comprising 21 animals in each.

The animals of group I provided distilled water and served as control. The rats of group II were provided aqueous extract of *Datura stramonium* seed @ 1000 mg/ kg body weight and group III were administered aqueous extract of *Datura stramonium* seed @ 2000 mg/ kg body weight orally for 90 days. The animals were maintained under standard managemental conditions and were provided feed and water *ad libitum*. All the experimental procedures, housing and management of the rats were strictly carried out according to the recommendations and approval of the Institutional Animal Ethics Committee (IAEC) as per the guidelines set forth by committee for the purpose of control and supervision of experiments on animals (CPCSEA). All the groups of rats were observed daily for clinical signs. The feed intake was recorded daily during the entire period of study. Body weight of all the experimental rats of group I, II and III was recorded at weekly intervals of experimentation. The blood samples were collected from retro-orbital plexus in rats of group I, II and III on day 30, 60 and 90 using micro-capillary tubes in vial containing EDTA @ 2 mg/ ml of blood as an anticoagulant for the study of Hemoglobin by cyanmet hemoglobin method using commercially available kit (Span Diagnostic Ltd) and the values were expressed in gm/dl, PCV, Total erythrocyte count, Erythrocytic indices (MCV, MCH, MCHC), Total leukocyte count, Differential leukocyte count method as described by [6] etc. The blood samples were collected from retro-orbital plexus on day 30, 60 and 90 in the rats of group I, II and III using micro-capillary tubes in 5.0 ml vacutainer containing heparin as anticoagulant. Heparinised blood sample were centrifuged at 2000 rpm for 15 min. Plasma was separated, transferred into plastic vial and stored at -20ºC for further analysis. The biochemical parameter studies *viz*. ALT, AST, Total bilirubin, Urea, Uric acid, Creatinine, Total cholesterol, Glucose, Total proteins, Albumin, Globulins and Alkaline phosphatase were done in rats of different groups at various intervals using standard diagnostic kits (Span Diagnostic Ltd., Surat).

All the data collected were statistically analyzed for arriving at meaningful conclusion. The quantitative data of feed intake, body weight, haematological observations, biochemical observations and Acetylcholinesterase estimation parameters were analyzed by Duncan's multiple range tests [7].

Results

All the animals of group-I (control) were active, bright with perfect vision and reflexes and did not show any clinical symptom of sickness throughout the experimental period. The feed and water intake was normal and were increasing with the advancement of experimentation. In the rats group-II revealed observable clinical signs following daily oral administration of *Datura stramonium* seed extract @1000 mg/kg body weight on 15 days post administration. The overt clinical signs were depression, loss of appetite, dilatation of pupils (mydriasis), increased thirst etc.

The rats of group-III showed clinical signs after 4th day of *Datura stramonium* seed extract administration @ 2000 mg/kg body weight. The clinical signs were restlessness and excitation for a longer period and this excitation were shifted toward the state of depression. Quarantine habit was usually observed in all the intoxicated rats. The examination of eye showed dilatation of the pupil. The depression prevailed in these rats throughout the study. The animals were less interested in food but water intake was increased. The other observed signs were dry skin, tachycardia and hyperthermia, dizziness, nervousness, muscular twitching in advance stage of the study. After 70 days of experimentation, the rats of group-III markedly showed anorexia, reduced water intake, defecation and urination, dry mucus membrane and disturbances in locomotion.

Mean value of feed intake are summarized in table-1. The feed intake was highly significant decrease from 31st day onward in rats of group-II and 16th day onwards in rats of group-III

Table 1: Mean values of feed intake in the rats of Group-I, Group-II and Group-III at various intervals.

Intervals	No. Of animals	feed intake (gm/day)		
		G-I	G-II	G-III
1-15 days	21	520.31±11.48^{a}	507.4±11.62^{a}	495.33±12.71^{a}
16-30 days	21	623.07±13.35^{b}	592.53±14.77^{b}	551.47±13.55^{a}
31-45 days	14	451.07±14.12^{b}	413.33±13.16^{a}	383.47±10.68^{a}
46-60 days	14	470.06±9.97^{c}	354.75±10.40^{b}	350.98±11.20^{a}
61-75 days	7	255.07±8.97^{c}	156.60±7.81^{b}	121.43±5.63^{a}
76-90 days	7	264.20±8.55^{c}	132.74±6.85^{b}	91.67±4.02^{a}

**The values with different superscript in the column are differing significantly in between the groups and between intervals.

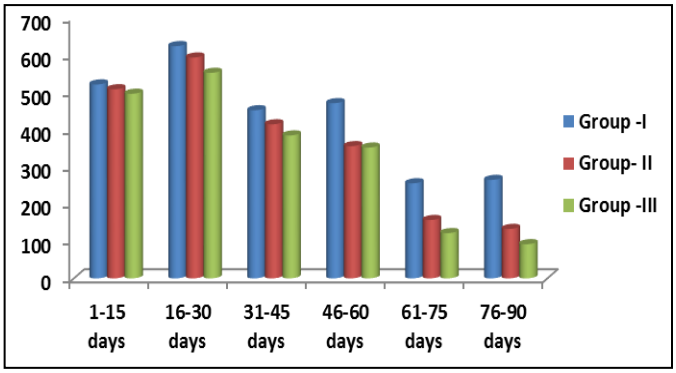

Fig 1: Effect on feed intake in the rats of Group-I, Group-II and group-III at different interval

Mean value of body weight of rats are summarized in table-2. The mean values of body weight of the rats of all the groups did not differ significantly up to 45 days of experimentation. In the rats of group-III, the statistical analysis revealed onset of significant (P<0.05) reduction in the body weight from day 52 and onwards. In the rat of group-II onset of the statistical analysis revealed significant (P< 0.05) reduction in the body weight were seen at 75 days to onwards respectively as compared to the group-I (control). In the rats of group-II and III the statistical analysis revealed onset of significant (P<0.05) reduction in body weight from day75 and 52 and onwards.

Table 2: Mean values of body weight in the rats of Group-I, Group-II and Group-III at different intervals.

DPF	No. of Animals	Body Weights (in grams)		
		G-I	G-II	G-III
0 days	7	104.71±4.73[a]	105.71±5053[a]	105.43±5.62[a]
7 days	7	119.57±4.31[a]	114.86±5.41[a]	112.42±5.67[a]
15 days	7	131.43±4.41[a]	124.57±5.96[a]	119.86±5.73[a]
21 days	7	141.00±5.07[a]	134.14±6.49[a]	128.29±5.69[a]
30 days	7	153.71±5.35[a]	145.71±5.56[a]	136.14±5.57[a]
37 days	7	162.57±5.25[a]	153.57±5.90[a]	144.00±5.74[a]
45 days	7	172.14±5.67[a]	162.14±5.70[a]	151.71±5.74[a]
52 days	7	183.28±6.93[b]	170.57±5.93[ab]	157.57±5.72[a]
60 days	7	197.28±9.2[b]	180.14±7.02[ab]	166.14±6.10[a]
67 days	7	206.28±8.52[b]	191.86±7.06[ab]	176.85±6.99[a]
75 days	7	219.86±8.49[b]	195.57±5.54[a]	186.85±7.68[a]
82 days	7	233.28±8.97[c]	201.27±5.92[b]	187.14±6.92[a]
90 days	7	248.14±9.89[c]	205.43±6.68[b]	185.71±6.55[a]

**The values with different superscript in the rows are differing significantly in between the groups and between intervals

Mean value of haemoglobin, PCV, TEC, TLC, MCV, MCH and DLC are summarized in table-4 and table-5 and figure 1. The mean values of Hb and PCV revealed highly significant decrease on day 30, 60 and 90 in group-III and on day 90 in group-II. TEC were highly significant decrease at all intervals in group-III and 60 and 90th day in group-II. Erythrocyte indices in the rats of group III revealed significant increase in the mean values of MCV on 30th and 60th day of trial. The TLC values revealed highly significant leucopenia on 30, 60 and 90 day post feeding in rats of group-III and 60th and 90th days in group-II. There was highly significant neutrophilia at 30th, 60th and 90th day in the rats of group-III and on 60th and 90th day of the experimentation period in the rats of group-II. There was highly significant lymphocytopenia at 30th, 60th and 90th days of experimentation in the rats of group-III and on 90th day of experimentation in the rats of group-II. The mean values of eosinophil and basophil did not reveal significant differences in the mean values of eosinophil in the rats of group-I and group-II respectively as compared to control group

Table 3: Mean values of Hb, PCV, TEC, MCV, MCH and MCHC in the rats of GroupI,Group-II and Group-III at various intervals

DPF	Groups	No.of rats	Haematological parameters					
			Hb (gm/dl)	PCV (%)	TEC (Million/mm^3)	Erythrocyte indices		
						MCV(FI)	MCH(Pg)	MCHC(gm/dl)
30	G-I	7	16.03±0.34[d]	47.64±1.30[d]	9.50±0.36[e]	50.26±0.71[a]	16.95±0.43[a]	33.70±0.55[a]
	G-II	7	15.94±0.56[cd]	46.07±1.47[cd]	8.49±0.2[cde]	51.45±0.93[abc]	17.81±0.70[ab]	34.75±1.43[a]
	G-III	7	13.68±0.72[bc]	41.07±2.18[bc]	8.01±0.49[bcd]	54.20±0.49[cd]	17.17±0.53[a]	33.33±0.46[a]
60	G-I	7	15.14±0.48[cd]	46.06±1.63[cd]	9.08±0.35[de]	50.73±0.33[ab]	16.69±0.15[a]	32.89±0.18[a]
	G-II	7	13.78±0.73[bc]	40.71±1.85[bc]	7.85±0.45[bc]	52.06±0.89[abcd]	17.58±0.28[ab]	33.77±0.30[a]
	G-III	7	12.12±0.68[ab]	37.21±2.07[b]	6.98±0.49[ab]	53.70±1.14[cd]	17.52±0.47[ab]	32.59±0.26[a]
90	G-I	7	15.25±0.64[cd]	46.03±1.86[cd]	9.09±0.43[de]	50.75±0.51[ab]	16.81±0.18[a]	33.13±0.18[a]
	G-II	7	12.49±0.72[b]	37.38±1.90[b]	7.11±0.38[ab]	53.23±0.83[bcd]	17.59±0.48[ab]	33.04±0.61[a]
	G-III	7	10.65±0.39[a]	31.78±1.18[a]	6.30±0.22[a]	50.77±1.05[ab]	16.90±0.30[a]	33.51±0.34[a]

Table 4: Mean values of TLC and DLC (Neutrophil, Lymphocyte, Monocyte Eosinophil and Basophil) in rats of various groups at different intervals

DPF	Gps	No	Haematological parameters					
			TLC(Thousand/mm^3)	Differential leukocyte count				
				Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
30	G-I	7	14.28±0.39[f]	22.14±1.76[a]	72.86±1.98[d]	3.28±0.42[a]	1.57±0.2[a]	0.14±0.14[a]
	G-II	7	13.65±0.24[def]	26.43±2.04[ab]	67.29±2.28[bcd]	4.29±0.78[a]	1.57±0.2[a]	0.43±0.20[a]
	G-III	7	13.03±0.28[cde]	30.86±1.89[bc]	62.86±1.87[ab]	4.0±0.49[a]	1.71±0.28[a]	0.57±0.20[a]
60	G-I	7	13.98±0.44[ef]	23.28±1.94[a]	70.0±2.31[cd]	4.28±0.77[a]	2.14±0.5[a]	0.29±0.18[a]
	G-II	7	12.72±0.43[bcd]	29.86±1.72[bc]	65.29±2.50[bc]	3.14±0.74[a]	1.14±0.4[a]	0.57±0.20[a]
	G-III	7	11.96±0.39[abc]	33.14±1.62[cd]	60.86±1.82[ab]	4.43±0.65[a]	1.28±0.42[a]	0.28±0.18[a]
90	G-I	7	13.89±0.47[def]	21.43±2.38[a]	73.14±2.77[d]	3.57±0.48[a]	1.29±0.36[a]	0.57±0.20[a]
	G-II	7	11.83±0.45[ab]	33.43±2.11[cd]	61.29±2.5[ab]	3.71±0.56[a]	1.14±0.34[a]	0.42±0.20[a]
	G-III	7	11.10±0.29[a]	37.28±1.68[d]	56.86±1.92[a]	4.28±0.42[a]	1.28±0.18[a]	0.28±0.18[a]

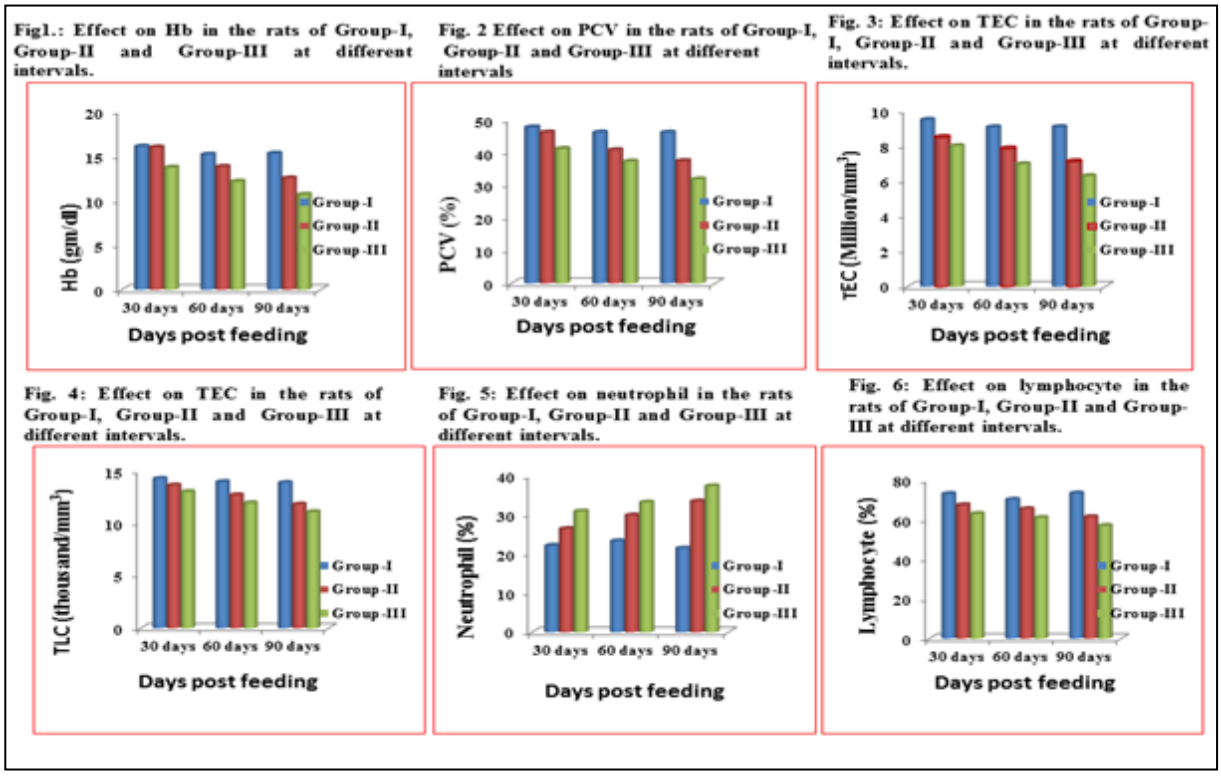

Fig 2: Mean value of hematological parameters are summarized

Mean value of ALT, AST, Total bilirubin, urea, uric acid, Creatinine, Total cholesterol, Glucose, Total proteins, Albumin, Globulins and Alkaline phosphatase are summarized in table-5 and table-6. The ALT values were increasing gradually but found statistically non significant in toxicity groups. The mean value of AST showed highly significant increase in rats of group-II on day 90 and in the rats of group-III on day 60 and 90. The values of total bilirubin and urea depicted highly significant increase in the rats of group-II on day 90 and in rats of group-III on day 60 and 90. The plasma uric acid revealed highly significant increase in the mean value on day 90 in rats of group-II and III. The mean value of plasma creatinine was highly significant increase on day 60 and 90 in group-II and on day 30, 60 and 90 in the rats of group-III. There was highly significant increase in cholesterol on day 90 in rats of group-II and on day 30, 60 and 90 in rats of group-III. The mean values of plasma glucose gradually increased in the rats of group-II and group-III at intervals of 30, 60 and 90 days post feeding, respectively but the statistical analysis did not show significant variation in between the groups and in between the intervals. The mean value of total proteins was highly significant reduced in the rats of group-III on day 60 and 90. However, the mean value of albumin was highly significant decrease on day 60 and 90 in group-II and on day 30, 60 and 90 in the rats of group-III. The globulin in the blood did not show any significant variation in all the groups. There was highly significant increase in the mean values of alkaline phosphatase in the rats of group-II on day 60 and 90 and in the rats of group-III on day 30, 60 and 90.

Table 5: Mean values of ALT, AST, Total bilirubin, urea, uric acid and Creatinine in the Rats of various groups at different intervals.

DPF	Groups	Numbers	Biochemical parameters in plasma					
			ALT (IU/L)	AST (IU/L)	Total bilirubin(mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
30	G-I	7	38.64±3.14^{a}	114.67±6.87^{abc}	0.28±0.05 ^{a}	38.89±3.02 ^{a}	2.59±0.35 ^{a}	0.39±0.07 ^{a}
	G-II	7	40.92±3.80 ^{a}	110.37±7.03 ^{a}	0.38±0.08 ^{a}	43.39±4.96 ^{a}	2.64±0.37 ^{a}	0.66±0.06^{ab}
	G-III	7	43.82±4.5 ^{a}	106.02±6.14 ^{a}	0.39±0.04^{ab}	47.09±4.99^{ab}	2.71±0.37 ^{a}	0.81±0.06^{b}
60	G-I	7	37.13±4.3 ^{a}	112.14±6.02^{ab}	0.30±0.08 ^{a}	40.21±3.87 ^{a}	2.55±0.35 ^{a}	0.39±0.04 ^{a}
	G-II	7	44.71±3.81 ^{a}	125.53±8.73^{abcd}	0.43±0.03^{abc}	48.15±4.95 ^{ab}	2.91±0.31 ^{a}	0.95±0.06^{b}
	G-III	7	48.62±4.3 ^{a}	137.39±7.8^{cd}	0.56±0.06^{bc}	58.73±3.17^{b}	3.35±0.39^{ab}	1.37±0.07^{c}
90	G-I	7	42.43±3.48 ^{a}	112.46±5.50^{ab}	0.29±0.03 ^{a}	39.95±3.52 ^{a}	2.63±0.33 ^{a}	0.40±0.04 ^{a}
	G-II	7	49.88±4.22 ^{a}	134.12±9.2^{cd}	0.60±0.08^{c}	71.96±4.70^{c}	3.99±0.28^{bc}	1.63±0.09^{c}
	G-III	7	55.56±4.78 ^{a}	140.43±7.14^{d}	0.79±0.06^{d}	83.86±4.98 ^{c}	4.78±0.34^{c}	2.91±0.26^{d}

Table 6: Mean values of Total cholesterol, Glucose, Total proteins, Albumin, Globulins and Alkaline phosphatase in the rats of various groups at different intervals

DPF	Groups	Numbers	Biochemical Parameters in plasma					
			Total cholesterol (mg/dl)	Glucose (mg/dl)	Total proteins (mg/dl)	Albumin (mg/dl)	Globulins (mg/dl)	Alkaline Phosphatase (IU/L)
30	G-I	7	48.48±3.86^{a}	111.61±7.61^{a}	6.85±0.32^{c}	4.61±0.088^{d}	2.24±0.30^{a}	228.97±7.82^{a}
	G-II	7	53.14±3.66^{ab}	116.21±8.08^{a}	6.54±0.38^{bc}	4.44±0.08^{cd}	2.09±0.39^{a}	240.21±7.03^{ab}
	G-III	7	58.48±3.11^{bc}	119.19±8.31^{a}	6.18±0.36^{bc}	4.22±0.09^{c}	1.96±0.34^{a}	258.41±8.83^{bc}
60	G-I	7	47.71±2.42^{a}	113.73±7.0^{a}	6.94±0.34^{c}	4.70±0.12^{d}	2.24±0.39^{a}	225.29±6.26^{a}
	G-II	7	57.24±3.01^{abc}	121.24±6.90^{a}	6.26±0.35^{bc}	4.27±0.08^{c}	1.99±0.32^{a}	260.16±5.82^{bc}
	G-III	7	65.62±2.67^{cd}	128.13±8.37^{a}	5.74±0.32^{a}	3.85±0.06^{b}	1.89±0.32^{a}	281.27±7.28^{c}
90	G-I	7	49.24±2.15^{ab}	115.16±7.33^{a}	6.89±0.28^{c}	4.63±0.09^{d}	2.26±0.29^{a}	231.49±2.29^{a}
	G-II	7	71.90±2.72^{d}	132.36±7.18^{a}	5.89±0.29^{abc}	3.90±0.15^{b}	1.99±0.28^{a}	334.35±9.38^{d}
	G-III	7	82.38±4.27^{e}	140.43±7.57^{a}	5.15±0.32^{a}	3.29±0.14^{a}	1.86±0.28^{a}	388.01±8.91^{e}

Table 7: Mean values of Total cholesterol, Glucose, Total proteins, Albumin, Globulins and Alkaline phosphatase in the rats of various groups at different intervals

DPF	Groups	Numbers	Biochemical Parameters in plasma					
			Total cholesterol (mg/dl)	Glucose (mg/dl)	Total proteins (mg/dl)	Albumin (mg/dl)	Globulins (mg/dl)	Alkaline Phosphatase (IU/L)
30	G-I	7	48.48±3.86^{a}	111.61±7.61^{a}	6.85±0.32^{c}	4.61±0.088^{d}	2.24±0.30^{a}	228.97±7.82^{a}
	G-II	7	53.14±3.66^{ab}	116.21±8.08^{a}	6.54±0.38^{bc}	4.44±0.08^{cd}	2.09±0.39^{a}	240.21±7.03^{ab}
	G-III	7	58.48±3.11^{bc}	119.19±8.31^{a}	6.18±0.36^{bc}	4.22±0.09^{c}	1.96±0.34^{a}	258.41±8.83^{bc}
60	G-I	7	47.71±2.42^{a}	113.73±7.0^{a}	6.94±0.34^{c}	4.70±0.12^{d}	2.24±0.39^{a}	225.29±6.26^{a}
	G-II	7	57.24±3.01^{abc}	121.24±6.90^{a}	6.26±0.35^{bc}	4.27±0.08^{c}	1.99±0.32^{a}	260.16±5.82^{bc}
	G-III	7	65.62±2.67^{cd}	128.13±8.37^{a}	5.74±0.32^{a}	3.85±0.06^{b}	1.89±0.32^{a}	281.27±7.28^{c}
90	G-I	7	49.24±2.15^{ab}	115.16±7.33^{a}	6.89±0.28^{c}	4.63±0.09^{d}	2.26±0.29^{a}	231.49±2.29^{a}
	G-II	7	71.90±2.72^{d}	132.36±7.18^{a}	5.89±0.29^{abc}	3.90±0.15^{b}	1.99±0.28^{a}	334.35±9.38^{d}
	G-III	7	82.38±4.27^{e}	140.43±7.57^{a}	5.15±0.32^{a}	3.29±0.14^{a}	1.86±0.28^{a}	388.01±8.91^{e}

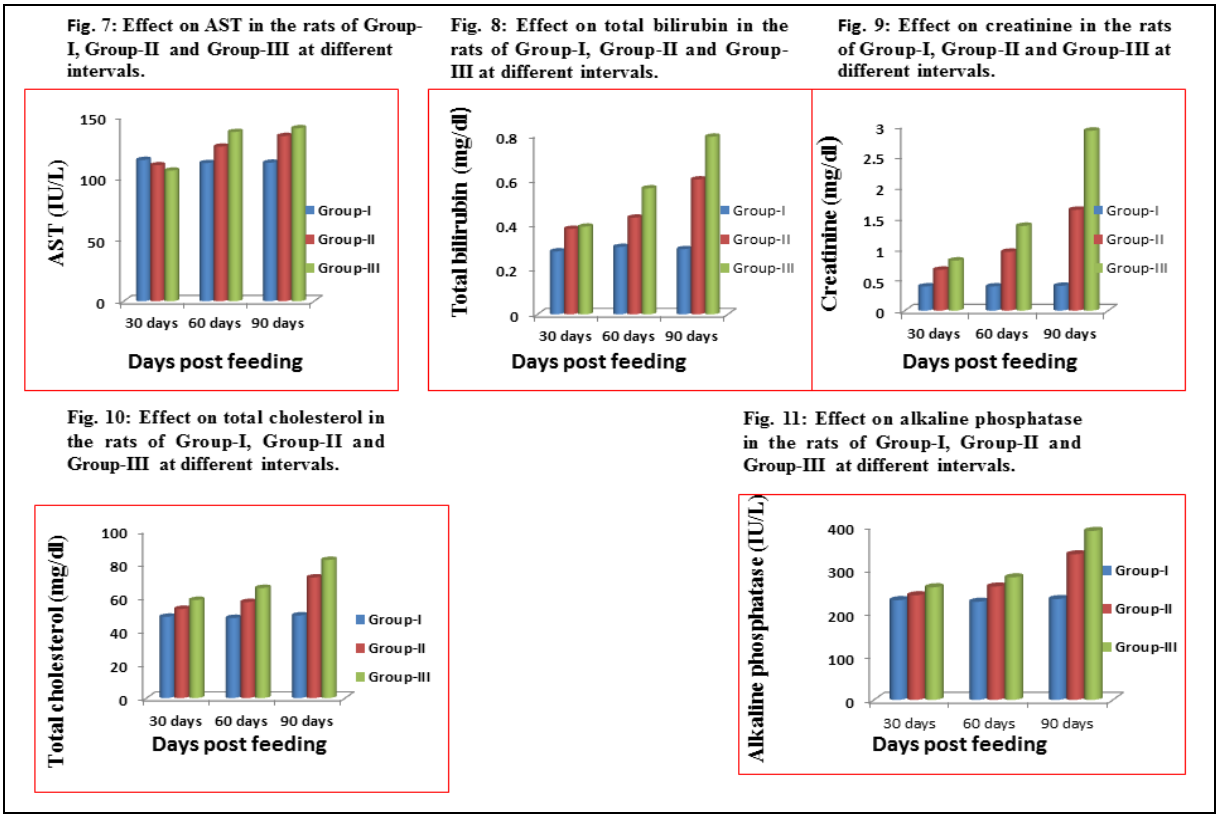

Fig 3: Mean value of biochemical parameters are summarized

Discussion

The present experimental study was conducted to observe the clinical, clinico-haematological and biochemical effects of *Datura stramonium* seed extract in experimental rats. The general clinical symptoms noticed in all the toxicity groups in the present study were depression, reduced appetite, and dilatation of pupils (mydriasis), and increased thirst, restlessness and excitation for a longer period which shifted toward the state of depression, quarantine habit, increased water intake, dry skin, tachycardia and hypothermia, dyspnoea, dizziness, nervousness, muscular tremor, defecation and urination, dry mucus membrane and

disturbances in locomotion, anuria in advance stage of the study. More or less similar clinical symptoms have been reported in rats [8, 9, 10, 11], ruminants [12], rabbits [13] and dogs [14, 15].

The increased heart rate in the present study confirms the finding of other workers [16, 17, 18] which may be due to removal of the parasympathetic components of vagal block.

The dyspnoea observed in the rats of toxicity groups may be a sequel of severe congestion in the lungs with variable degree of lesions of pneumocytocarcinoma in the rats of toxicity groups at later intervals of experimentation. The revelation of dilatation of pupils in the present study due to *Datura* intoxication has been reported to be a common symptom in animals [19, 20].

The mean values of feed intake revealed highly significant decrease on day 31 and onwards in rats of group-II and day 16 onwards in rats of group-III in the present study. Similar results have also been reported in pigs [21, 22, 23], horses [24] and poultry [25, 26, 27, 28]; Contrary to the observation of decreased feed intake in the present study, no significant effect on feed consumption in rats administered @ 50 mg/kg and 200 mg/kg *Datura stramonium* leaves extract was reported by [11]. The state of depression precipitated by scopolamine alkaloids of *Datura* seed in the present study may be reasonably associated with decreased interest in food intake [29].

The rats of group II and III, revealed onset of significant reduction in the body weight from day 75 and 52 and onwards, respectively. Similar results in Datura poisoning have been reported in rats [30, 31], Pigs [27, 28, 32, 33]. The growth retardation is attributed to the decreased protein synthesis and disturbances in metabolic function [27] and impairment of the basal metabolic activities [28, 34] suggested that the reduced body weight gain is due to presence of toxic alkaloids causing disturbance in growth and increased renal dysfunction resulting into loss of protein.

The mean values of haemoglobin, PCV and TEC indicated onset of moderate to marked anaemia in the toxicity groups. It appears that prolonged use of *Datura stramonium* seeds are toxic to proliferating haemopoietic tissue leading to development of significant anaemia in the rats. The findings of the present study are contradictory to the observation in horses [24, 30]. However, no significant haematological change after oral administration of *Datura stramonium* was observed in rats [11]) and chicks [28].

In present study the statistical analysis showed highly significant increase in the mean values of MCV on 30th and 60th day of trial in rats of group-III as compared to group-I. Increase in MCV in the present study suggested premature release of erythrocytes in the circulation from bone marrow leading to macrocytic anaemia as a compensatory mechanism. In contrast, no significant changes in MCV following oral administration of *Datura stramonium* were observed in rats [35] and chicks [28].

The mean values of TLC were revealed highly significant decrease on 30th, 60th and 90th days in rats of group-III and on 60th and 90th day in the rats of group-II as compared to group-I. The findings of the present study are contradictory to the observation where increased in the total leukocyte counts were observed by Binev *et al*., (2006b) in horses. However, no significant change in TLC after oral administration of *Datura stramonium* was observed in rats [35] and chicks [28].

The mean values of neutrophil and lymphocyte revealed highly significant neutrophilia and lymphocytopenia at variable intervals in toxicity groups. Similar results have been reported in the horse [24]. However, no significant change in the count of neutrophil and lymphocyte following oral administration of *Datura stramonium* were observed in rats [35] and chicks [28].

The mean values of plasma AST were depicted highly significant increase in the values of AST in the rats of group-II on day 90 and in the rats of group-III on day 60 and 90 as compared to group-I. Similar findings have been reported in Datura stramonium seed extract toxicity in broilers [27] rats [30], chicks [35] and horses [24]. Contrary to the observation of the present study [11], did not observe any significant changes in the values of AST in rats after oral administration of *Datura stramonium* leaves extract.

The mean values of total plasma bilirubin were revealed highly significant increase in the mean values of total bilirubin in rats of group-II on day 90 and in the rats of group-III on day 60 and 90 as compared to group-I. At 90 days of experimentation there was highly significant increase in the mean values of total bilirubin in between the toxicity groups. Similar findings have been reported in Datura stramonium seed extract toxicity in horses [24], pigs [22] and rats [10]. Contrary to the observation of total bilirubin in the present study [11], did not observe any significant change in the total bilirubin after oral administration of *Datura stramonium* leaves extract in rats.

The mean values of plasma urea levels were revealed highly significant increase in the rats of group-II at the intervals of 90 days post feeding and in the rats of group-III at intervals of 60 and 90 days post feeding as compared to group-I (control). Similar findings have been reported in Datura stramonium seed extract toxicity in rats [10] sheep and goats [36] and pigs [22]. The findings of the present study are contradictory to the observation in rats where no changes in plasma urea level was observed as a result of oral administration of *Datura stramonium* leaves extract @ 200 mg/kg body weight [11].

The mean values of plasma uric acid were indicated highly significant increase in the mean values of plasma uric acid in rats of group-II and group-III on day 90. Similar findings have been reported by [22] in pigs. In contrast [28]) reported that *Datura stramonium* seed toxicity in chicks has no significant effect on serum uric acid level.

The mean values of plasma creatinine were revealed highly significant hypercretinemia in rats of group-II on day 60 and 90 and in the rats of group-III on day 30, 60 and 90. The estimation of creatinine in blood has been reported to be an index for determining renal function [37]. The observation of increased value of plasma creatinine in the present study amply suggested that the *Datura stramonium* seed extract induces nephrotoxic effects which are further supported by presence of histopathological lesions in kidneys described under the section of pathomorphology (vide infra). The findings in the present study are in close agreement with the induced *Datura stramonium* seed extract toxicity in rats [11] and [10] and Broilers [27]. Contrary to the present observation, [22] reported that *Datura stramonium* seed toxicity in pigs has no significant effect on serum creatinine level.

Increased values of plasma urea, uric acid and creatinine suggest, beyond any doubt, induction of kidney damage by the prolonged administered *Datura stramonium* aqueous seed extract.

The mean values of total plasma cholesterol were depicted highly significant increase in the mean values of total cholesterol in the rats of group-II on day 90 and in the rats of group-III on day 30, 60 and 90 as compared to group-I

(control). At 90 days of experimentation there was highly significant increase in the mean values of cholesterol in both the toxicity groups. Similar findings have also been reported by [22] in pigs. Contrary to the observation of total cholesterol in the present study, [11] did not observe any significant change in the values of total cholesterol after oral administration of *Datura stramonium* leaves extract in rats. However, decrease in the mean values of total cholesterol following induction of *Datura stramonium* toxicity has been reported in rats [11] and chicks [28].

The mean values of plasma glucose gradually increased in the rats of group-II and group-III at intervals of 30, 60 and 90 days post feeding, respectively albeit non significant. Similar findings have also been reported by [11] in rats. Contrary to the present observation of hyperglycaemia due to *Datura stramonium* intoxication has been reported in pig [22] and horses [24].

The mean values of total plasma protein were indicated marked hypoproteinaemia in group-III on 60 and 90 days of intervals. The decrease in the level of plasma protein may be attributed to increase catabolism of proteins, loss of plasma protein in urine, hepatic and renal dysfunctions as also observed histopathologically in the liver and kidneys in this study. The observation of hypoproteinaemia due to *Datura stramonium* seed toxicity in this study confirms the finding of the *Datura stramonium* toxicity in rats [10], chicks [28], broilers [27], sheep and goats [36] and horses [24]. In contrast to the present observation, [11] reported no significant effect on the level total plasma proteins in rats following *Datura stramonium* seed intoxication.

There was highly significant decrease in the concentrations of plasma albumin in the rats of group-II on day 60 and 90 and in the rats of group-III on day 30, 60 and 90. At 60 and 90 days of experimentation there was highly significant reduction in the mean values of albumin in both the toxicity groups. The hypoalbuminaemia in the present study is in close agreement with the observation in rats [30], chicks [28] and broilers [27]. Contrary to the present observation, [24] reported that the *Datura stramonium* toxicity in horses produced no significant effect on concentration of serum albumin.

There was highly significant decrease in the concentration of alkaline phosphatase on day 60 and 90 in the rats of group-II and on day 30, 60 and 90 in the rats of group-III. At 90 days of experimentation there was highly significant increase in the mean values of alkaline phosphatase in both the toxicity groups. Similar findings have also been reported in [30] and broilers [27].

It is concluded that seed of Datura *stramonium* have a potent toxic ability and produce fatal effect on feed intake and body weight gain. Feed intake and body weight gain decreases was due to consumption of dhatura seed and also it causes alteration of clinico-haematobiochemical parameter of in rats.

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