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Subacute toxicopathological evaluation of acephate toxicity and it's amelioration by *Swertia chirata* in male Wistar rats: haemato-biochemical studies

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Abstract
Study on subacute toxicopathological evaluation of Acephate and it's amelioration by *S. chirata* was conducted in male Wistar rats. Thirty male rats were divided into 5 groups each comprising of 6 rats viz. (group I and III) kept as a control and plant control, group II (Acephate toxicated rats) & group IV and V (toxicated with Acephate and treated with *S. chirata*) and on 14th and 28th day haematobiochemical parameters were studied.
Rats of group II and IV revealed significant reduction in Hb, TEC, PCV, TLC and delay the blood clotting time, where as, non-significant variation were observed in DLC.
There was significant increase in serum glucose levels, AST, ALT, ALP, serum cholesterol, serum urea nitrogen in rats of group II and IV. Significant reduction in the levels of STP, serum sodium, serum potassium and serum AchE in rats of group II and IV was noted.
These altered values showed improvement due to *S. chirata* (group V).

Keywords: subacute acephate toxicity, Wistar rats, haemato-biochemical, *Swertia chirata*

Introduction
Man is the ultimate consumer of pesticide residues. Through fodder, water, air and other feed stuff pesticide residues reaches in animal and then through milk, meat, egg and other animal products accumulates in human beings. Pesticides are the most common xenobiotics present in the environment and causing toxicity. Prolong exposure of pesticides affected the normal functioning of different organ system of animals and produced many clinical effects (Azmi *et al.*, 2006 and Sharma and Singh, 2010) [3, 19].
Organophosphates (OPs) are toxic chemicals which are frequently used in agriculture to repel pests. Pesticides, such as acephate, have become a public health concern because pesticide exposure leads to harmful effects in human metabolism, such as hyperglycemia, lipid metabolism dysfunction, DNA damage, increased oxidative stress and cancer, which are rapidly growing epidemics and which lead to increased morbidity and mortality rates and soaring health-care costs (Costa, 2006) [5]. Acephate is a potent neurotoxic, mutagenic, carcinogenic and cytotoxic compound (Singh and Jiang, 2002) [21].
Recently, In Vidarbha region, Yawatmal district of Maharashtra state around 50 farmers and farm labours died and nearly 800 were hospitalized due to pesticide poisoning (Hindustan Times Yawatmal, 13th Oct, 2017). In past three years, 442 farmers and farm workers died after inhaling poisonous pesticides in India, a recent report by union health ministry revealed. Over 94% of the deaths were from Punjab (233). Whereas, the Maharashtra (183), Rajasthan (20), Karnataka (3), Tamilnadu (3) were other states that figured on the list (Hindustan Times, 22nd March, 2017).
In Ayurveda, *Swertia chirata* is used as antipyretic, anthelmintic, anti-inflammatory, anti-carcinogenic, anticholinergic, antioxidant, antimalarial, hypoglycemic, in mutagenicity laxative, in asthma and in leucorrhoea. In Indian medicinal system, chirata is used as remedy for bronchial asthma, liver disorders, chronic fever, anemia, stomachic and diarrhoea. In Yunani system the plant is used as astringent, tonics, stomachic, lessens inflammation, sedative to pregnant uterus and chronic fever (Kirtikar and Basu, 1984) [7]. The whole plants of *Swertia* are medicinal but roots are the most powerful parts.
Considering these facts, present study has been conducted to assess ameliorative effects of *Swertia chirata* against Acephate,

(O, S-Dimethyl Acetyl phosphoramidothioate) induced toxicity in Male Wistar rat to assess subacute toxicity of acephate and its amelioration by Swertia chirata through haematobiochemical studies.

Materials and Methods

Details of Experimental group of rat

A total of 30 male Wistar rats ageing about 4-6 weeks and approximately 140-150 gms of weights were divided into five groups each comprising of 6 rats. Group I (Healthy control) with normal feeding & watering, group II (Acephate tocicity) Acephate @ 34.4 mg/kg Bwt., with vehicle, group III (plant extract control) *Swertia chirata* aq. extract @ 100 mg/kg Bwt., group IV (Treatment I) Acephate @ 34.4 mg/kg Bwt., with vehicle+ *Swertia chirata* aq. extract @ 50 mg/kg Bwt., group V (Treatment II) Acephate @ 34.4 mg/kg Bwt., with vehicle+ *Swertia chirata* aq. extract @ 100 mg/kg Bwt., by oral gavage route. And were maintained for 28 days. The experimental male Wistar rats were procured from M/S Wockhardt Research Centre D4, M.I.D.C., Chikalthana Aurangabad (MS). Prior to experiment, all the rats were kept at laboratory condition for a period of 10 days for acclimatization. The animals were housed in polypropylene cages under controlled conditions. The animals were maintained under standard managemental conditions and provided with feed and water *ad-libitum* throughout experimental period. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol, (Approval no. IAEC/24/17 Date: 13/10/2017) which met the National guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Also, the Outline of Research Work has been approved by Board of Studies (Veterinary Pathology), MAFSU, Nagpur (M.S.) Resolution No. 02/17 Date: 05/01/2018.

Procurement of acephate pesticide and plant

Powder form of *Acephate* was procured from Department of Pesticide, VNMKV College, Parbhani. Dose of *Acephate* was calculated as 1/20th of LD_{50} (688 mg/kg) of male wistar rats (UPI 2010). *Acephate* powder was weighed and then it was dissolved in 1ml propyelene glycol per animal. This solution was used to induce toxicity in male Wistar rats.

Swertia chirata plant procured from the local market of Parbhani (MS) and powered by using electric grinder. Extraction was done by hot water extraction method. The whole plants (stem, leaves and roots) powder @ 250 gm was dissolved in 2 lit. of Distilled water and boiled till it makes a half of it i.e. 1 lit. of this plant solution. After that this solution was poured in a conical flask and cooled down at room temperature. With the help of muslin cloth and whatman filter paper no.42 this solution was filtered in other conical flask and the final *Swertia chirata* plant extraction was obtained to feed to experimental male Wistar rats by oral gavage.

Haematobiochemical studies

Blood samples were collected from the retro-orbital plexus from the rats in clean, dry and sterilized EDTA vials (for haematology) and in Ependroffs tube without anticoagulant (for serum separation) were carried out for all groups at 14th and 28th day of study, for the haemato-biochemicallogical studies. The serum samples were analysed for STP, serum glucose, serum sodium, serum potassium, serum AST, serum ALT, serum cholesterol, serum ALP, Serum urea nitrogen and Serum AchE by using Ambica Diagnostic Reagent Kits on Erba Clinical Autoanalyser.

Statistical analysis

The data generated from various parameters were statistically analyzed by Factorial Randomized Design (FRD) and Completely Randomized Design (CRD) using WASP. to know the statistical differences between means of various parameters at different intervals in each group, as per the methods described by Snedecor and Cochran (1994).

Results and Discussion

Haematological studies

Table 1 and Fig 1 dipicts the details of haematological parameters studied

All the haematological parameters of rats of healthy control group (Group I) and plant control group (Group III) showed within normal physiological limits.

In rats of toxicity control group (Group II) fed with acephate @ 34.4 mg/kg Bwt., for 28 days daily P.O. there was significant reduction in the haematological parameters such as Hb (14th day onwards); TEC (on 28th day); PCV (14th day onwards). Moreover, there was significant elevation in clotting time (14th day onwards) and TLC (on 28th day) and there were no alterations in DLC counts.

Rittenhouse *et al.,* (1972) [25]; Mishra (2014) [10] and Sharma *et al.,* (2015) [20] in their studies observed similar findings. Reduction in haemoglobin level might be due to significant reduction in body weight and serum total protein levels of rats. In the present study the findings of haematological alterations might be attributed to acephate induced stress, inhibition of Na+/K+ ATPase activity in cells, decreased eythopoiesis due to acephate or nutritional imbalance. The other reason might also be due to the toxic damage to the vital organs particularly spleen, liver, kidney and bone which are directly or indirectly related with haematopoieis (Rao and Vidyunmala, 2010) [13] which support the findings of this study.

In rats (Group IV fed with acephate @ 34.4 mg/kg Bwt.) and treated with *S. Chirata* (@ 50 mg/kg Bwt.) It was observed that altered haematological values by acephate toxicity were not improved in rats of this group at both the intervals of the study. However, clotting time was partially improved (at both the intervals) in rats in this group. There were no alterations in DLC Counts in rats of this group.

The rats of Group V (Treatment II) altered haematological parameters by acephate toxicity were significantly improved viz; Hb (at 14th day onwards); TEC (at 28th day); PCV (at 14th day onwards); TLC (on 28th day) and clotting time (at 14th day onwards) Whereas, DLC counts did not altered at both the intervals.

Turaskar *et al.,* (2013) [25] reported reduction in Hb concentration (42.12% of the control) in CCL_4 toxicity which improved after *S. chirata* ethanolic extract treatment (@ 200 mg and 400 mg/kg Bwt.), which supports the findings of the present study.

Biochemical studies

Table 2 and Fig 2 dipicts the details of biochemical parameters studied.

All the biochemical parameters of rats of healthy control group (Group I) and plant control group (Group III) showed within normal physiological limits.

There was significant increased in serum AST, ALT and ALP levels in rats of group II and group IV at 14th and 28th day

intervals of study. While, these values in rats of group III and V remained comparable with threats of control group. moreover, these values of group V were found significantly improved than the values in rats of group II and were comparable to values in rats of group I and group III, and indicating, ameliorative effects of aq. extract of *S. chirata* against acephate induced toxicity. Necrosis or membrane damage releases the enzymes such as AST, ALT and ALP and bilirubin and hence, can be measured in the serum. High levels of AST indicate liver damage. The rise in serum levels of AST attributed to damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. (Pereira *et al.,* 2013; Mishra 2014 and Sarah Nwozo *et al.,* 2015) [10, 12, 17].

Mean values of serum total protein were decreased significantly on 14th day onwards in rats of group II and IV. The STP values were improved in rats of group V and were found to be comparable with values in rats of healthy control group. Mean of STP values in male rats of group I and III remained statistically at par with respective STP values of control group. reduced STP levels could be due to reduction in feed intake which resulted into decrease in body weight (Sankhala 2007 and Mishra 2014) [10, 16].

There was significant increase in serum glucose level in rats of group II and IV on 28th day onward as compared to values of control group. Moreover, the mean values of serum glucose in rats of group III are comparable to healthy control group. Hyperglycemia observed in experimental rats due to acephate intoxication or inhibition of insulin secretion from β-cells. OP insecticides stimulate catecholamines that are known to inhibit insulin secretion by activation of α- receptor of pancreas (Mishra 2014) [10].

In rats of group II and group IV there was significant elevation in serum cholesterol on 14th day onwards and serum urea nitrogen on 28th day. The mean values of serum cholesterol and SUN in rats of group III were found comparable to healthy control group I. group V indicated significant improvement in mean values of serum cholesterol and SUN. Increase in SUN level might be due to renal injury in rats of acephate intoxication. (Thapar *et al.,* 2002 and Mishra 2014) [10, 24]. Cholsterol increases due to Liver is involved in lipid synthesis, metabolism, and transportation and changes in plasma lipid level could serve as a simple marker for assessing liver disorders These alterations in lipid profile might be attributed to the effect of fenitrothion on the permeability of hepatocyte cell membrane or blockage of the bile duct which reduces or stops cholesterol secretion into the duodenum Also, this could be attributed to increased hepatic synthesis and/or diminished hepatic degradation of lipids due to reduced lipoprotein lipase activity (Rasha *et al.,* 2016) [14].

There was significant decrease in the mean serum sodium and serum potassium in rats of group II and IV on 14th day onwards due to enhanced lipid peroxidation by free readicals in acephate intoxicated rats (Siraj *et al.,* 2010) [22]. group III were found comparable to healthy control group I. An improvement in mean values of sodium and serum potassium in rats of group V which suggested that the plant extract preserving the structural integrity of hepatocellular membrane, thus prevented enzymes leakage into circulation due to its hepatoprotective, nephroprotective, anti-oxidative, antidiabetic and immunomodulatory properties. (Chakravarti *et al.,* 1994; Anil Kumar *et al.,* 2012; Mukherjee *et al.,* 1997; Mandal *et al.,* 1992; Scartezzini and Speroni, 2000) [1, 4, 8, 11, 18].

The mean values of serum AchE found significantly decrease in rats of group II and IV at 14th and 28th day intervals of study. Moreover, the mean values of serum AchE in rats of group V remained comparable and significantly improved with the values of group I and III which indicating its ameliorative effect to minimizing the neurotoxic effects. The primary target site for the Ops compound is the Ach. The work of OPs is inhibition of the action of neurotransmitter AchE in the synaptic regions, thereby interfering with normal mode of nerve impulse propagation (Wang *et al.,* 2004 and Menozzi *et al.,* 2004) [9, 27].

Table 1: showing mean values of Haematological parameters of experimental male rats studied at 14th and 28th day of intervals of study14th and 28th day intervals of study

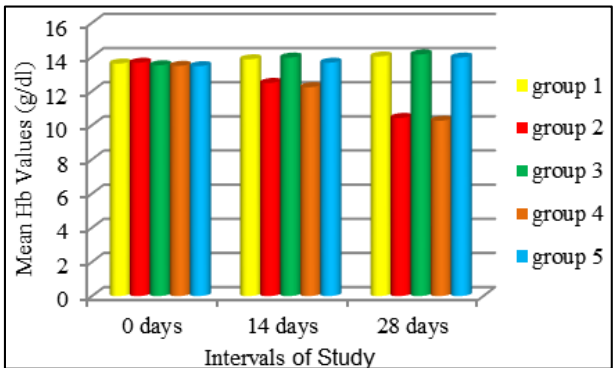
Haematological parameters								
Parameter	**Day**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**CD**	**Stati-tics**
Haemoglobin (g/dl)	14th	13.86a ± 0.18	12.48b ± 0.19	13.36a ± 0.28	12.23b ± 0.32	13.66a ± 0.32	0.789	S
	28th	14.03a ± 0.17	10.43b ± 0.21	14.15a ± 0.18	10.26b ± 0.30	13.96a ± 0.28	0.692	S
Total Erythrocyte Counts(10^6/cmm)	14th	7.04 ± 0.07	6.70 ± 0.17	7.05 ± 0.23	6.39 ± 0.26	6.91 ± 0.24	-	NS
	28th	7.06^a ± 0.04	5.87^b ± 0.23	7.25^a ± 0.18	5.78^b ± 0.28	7.11^a ± 0.14	0.562	S
Packed Cell Volume (%)	14th	34.3^a ± 0.48	31.1^b ± 0.53	34.5^a ± 0.90	30.8^b ± 0.61	34.1^a ± 1.01	2.167	S
	28th	35.1^a ± 0.58	28.9^b ± 0.53	36.1^a ± 1.10	28.7^b ± 0.73	34.7^a ± 0.89	2.334	S
Total Leukocytes Counts(10^3/cmm)	14th	9.37 ± 0.53	9.80 ± 0.39	9.55 ± 0.90	9.72 ± 0.76	9.30 ± 0.45	-	NS
	28th	9.98^b ± 0.64	12.03^a ± 0.24	9.86^b ± 0.80	11.01^{ab} ± 0.59	9.78^b ± 0.44	1.681	S
Clotting Time (Sec)	14th	54.18^c ± 0.93	68.16^a ± 0.70	54.93^c ± 1.04	64.66^b ± 0.61	54.66^c ± 0.49	2.282	NS
	28th	55.33^c ± 0.71	80.28^a ± 0.45	55.48^c ± 1.17	76.50^b ± 0.76	55.00^c ± 0.63	2.295	NS
Neutrophils Counts (%)	14th	22.83 ± 1.04	27.00 ± 0.44	27.00 ± 0.63	26.00 ± 0.57	27.33 ± 0.49	-	NS
	28th	28.83 ± 0.70	27.83 ± 0.60	28.00 ± 0.57	27.50 ± 0.67	27.33 ± 0.49	-	NS
Eosinophils Counts (%)	14th	3.33 ± 0.42	3.00 ± 0.36	2.16 ± 0.30	2.00 ± 0.36	2.00 ± 0.51	-	NS
	28th	3.16 ± 0.30	3.00 ± 0.57	2.16 ± 0.30	2.16 ± 0.47	2.66 ± 0.33	-	NS
Lymphocyte Counts (%)	14th	66.50 ± 0.84	67.83 ± 0.30	68.66 ± 0.91	69.50 ± 0.50	68.16 ± 0.79	-	NS
	28th	65.83 ± 0.90	66.16 ± 0.65	68.00 ± 0.77	68.00 ± 0.96	67.50 ± 0.71	-	NS
Monocyte Counts (%)	14th	2.00 ± 0.51	2.16 ± 0.30	2.16 ± 0.47	2.50 ± 0.34	2.50 ± 0.42	-	NS
	28th	2.16 ± 0.30	2.00 ± 0.36	2.16 ± 0.47	2.33 ± 0.42	2.50 ± 0.42	-	NS

*****Means bearing similar superscripts in column and rows do not differ significantly ($P < 0.05$)

Table 2: showing mean values of Biochemical parameters of experimental male rats studied at 14th and 28th day of intervals of study14th and 28th day intervals of study.

Biochemical parameters								
Parameter	**Day**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**CD**	**Statistics**
Serum Total Protein (gm/dl)	14th	$6.31^{a} \pm 0.17$	$5.80^{b} \pm 0.21$	$6.33^{a} \pm 0.13$	$5.79^{b} \pm 0.14$	$6.30^{a} \pm 0.13$	0.489	S
	28th	$6.86^{a} \pm 0.09$	$5.63^{b} \pm 0.20$	$6.81^{a} \pm 0.08$	$5.48^{b} \pm 0.15$	$6.80^{a} \pm 0.11$	0.396	S
Serum glucose (mg/dl)	14th	146.36 ± 1.55	151.65 ± 1.88	144.95 ± 2.08	149.88 ± 2.47	144.53 ± 2.65	-	NS
	28th	$147.9^{c} \pm 1.53$	$161.8^{a} \pm 1.85$	$146.52^{c} \pm 2.00$	$154.38^{b} \pm 2.58$	$146.41^{c} \pm 2.71$	6.362	S
Serum Sodium (mEq/L)	14th	$144.16^{a} \pm 1.73$	$137.56^{b} \pm 1.56$	$143.23^{a} \pm 0.83$	$136.70^{b} \pm 1.17$	$141.67^{a} \pm 0.93$	3.785	S
	28th	$144.05^{a} \pm 1.51$	$136.61^{bc} \pm 4.44$	$144.23^{a} \pm 1.21$	135.85c ± 1.63	$142.62^{ab} \pm 0.65$	6.730	S
Serum Potassium (mEq/L)	14th	$7.07^{a} \pm 0.34$	$5.56^{b} \pm 0.27$	$6.96^{a} \pm 0.28$	$5.50^{b} \pm 0.17$	$6.90^{a} \pm 0.16$	0.746	S
	28th	$7.18^{a} \pm 0.36$	$4.82^{d} \pm 0.43$	$7.14^{ab} \pm 0.31$	$4.66^{cd} \pm 0.18$	$6.71^{bc} \pm 0.17$	0.917	S
Serum AST (IU/L)	14th	$76.90^{bc} \pm 1.81$	$90.00^{a} \pm 1.14$	$76.91^{bc} \pm 3.55$	$82.81^{b} \pm 2.66$	$75.98^{c} \pm 1.26$	6.630	S
	28th	$79.71^{b} \pm 1.15$	$92.56^{a} \pm 1.43$	$78.65^{b} \pm 3.06$	$86.69^{a} \pm 2.71$	$76.73^{b} \pm 1.46$	6.158	S
Serum ALT (IU/L)	14th	$33.56^{b} \pm 2.39$	$70.56^{a} \pm 2.68$	$34.40^{b} \pm 3.54$	$64.99^{a} \pm 5.40$	$33.37^{b} \pm 1.02$	9.728	S
	28th	$34.58^{b} \pm 2.58$	$76.16^{a} \pm 1.69$	$35.69^{b} \pm 3.45$	$71.78^{a} \pm 1.25$	$34.17^{b} \pm 0.70$	6.325	S
Serum Cholesterol (mg/dl)	14th	$89.83^{c} \pm 1.13$	$107.5^{a} \pm 1.33$	$90.93^{c} \pm 0.54$	$103.9^{b} \pm 1.21$	$90.66^{c} \pm 0.84$	3.079	S
	28th	$91.33^{c} \pm 1.11$	$117.66^{a} \pm 1.40$	$91.83^{c} \pm 0.60$	$112.03^{b} \pm 1.49$	$90.93^{c} \pm 0.64$	3.256	S
Serum ALP (IU/L)	14th	174.32 ± 3.42	180.00 ± 3.10	174.40 ± 3.66	173.18 ± 1.41	171.68 ± 1.83	-	NS
	28th	$175.84^{bc} \pm 3.17$	$190.00^{a} \pm 2.13$	$175.20^{c} \pm 3.59$	$183.18^{ab} \pm 1.90$	$174.08^{c} \pm 1.98$	7.729	S
Serum urea nitrogen (mg/dl)	14th	$21.73b^{c} \pm 1.03$	$27.05^{a} \pm 0.27$	$21.48^{bc} \pm 0.75$	$23.01^{b} \pm 0.41$	$20.50^{c} \pm 0.46$	1.895	NS
	28th	$23.07^{b} \pm 0.70$	$31.63^{a} \pm 0.69$	$23.19^{b} \pm 0.44$	$30.56^{a} \pm 0.81$	$21.75^{b} \pm 0.50$	1.891	S
Serum AchE (µmol/L)	14th	$3.92^{a} \pm 0.29$	$2.10^{b} \pm 0.01$	$3.99^{a} \pm 0.27$	$2.08^{b} \pm 0.01$	$3.82^{a} \pm 0.33$	0.688	S
	28th	$3.94^{a} \pm 0.29$	$1.17^{b} \pm 0.05$	$3.98^{a} \pm 0.27$	$1.06^{b} \pm 0.01$	$3.83^{a} \pm 0.32$	0.686	S

*Means bearing similar superscripts in column and rows do not differ significantly ($P < 0.05$)



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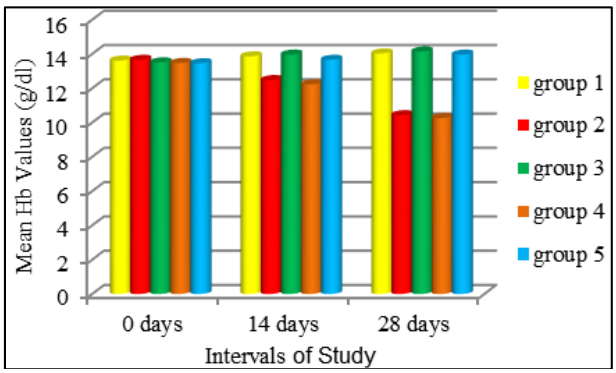

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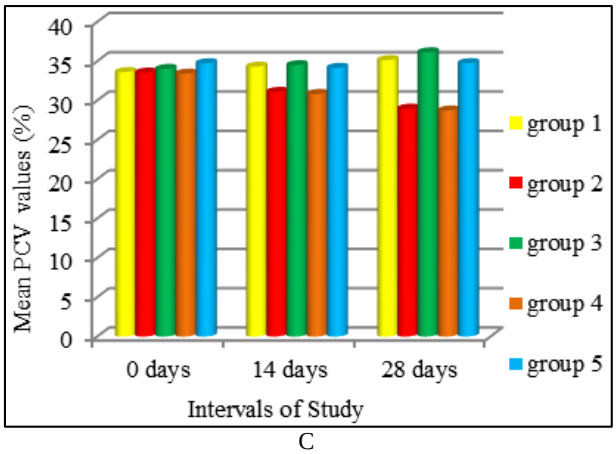

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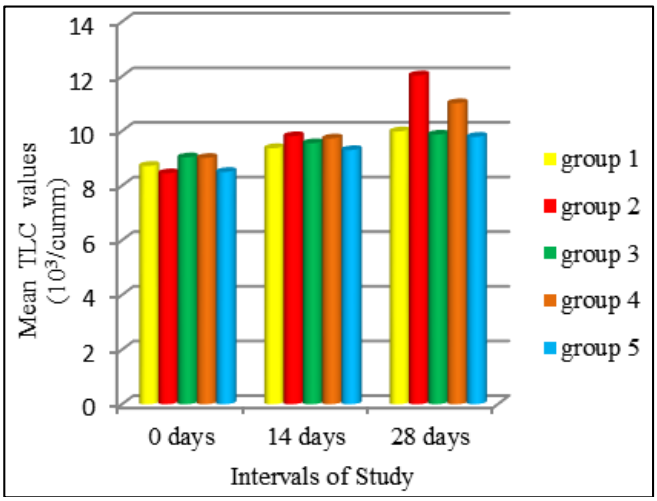

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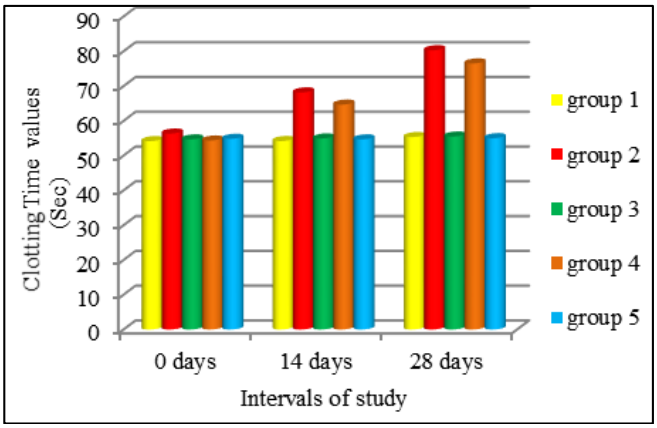
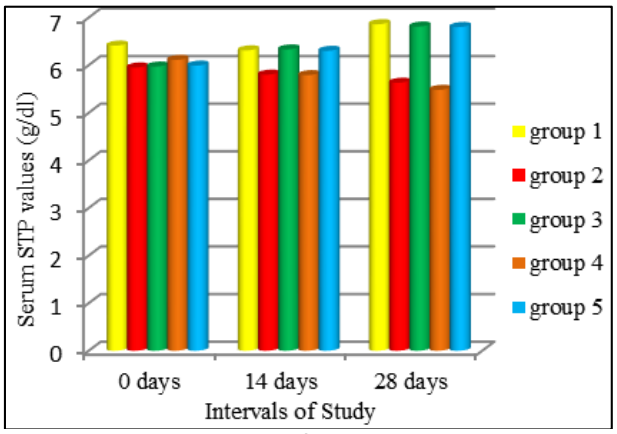
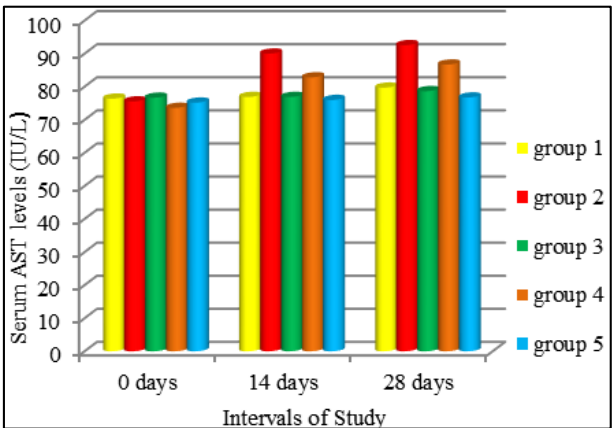


E

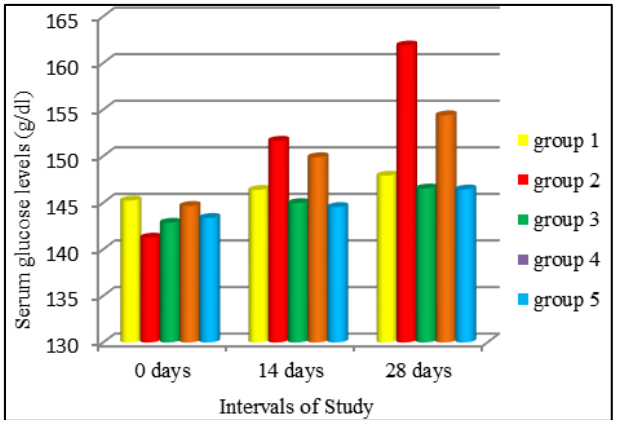
Fig 1: (a) Values of Haemoglobin in experimental male rats at different intervals of study. (b) Total Erythrocyte Counts in experimental rats at different intervals of study. (c) Mean values of Packed Cell Volume in experimental rats at different intervals of study.(d) Mean values of Total Leukocytes Counts (10^3/cumn) in experimental rats at different intervals of study. (e) Mean Values of Clotting Time (Sec) in experimental male rats at different intervals of study.


Serum STP values (g/dl)
0 1 2 3 4 5 6 7
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

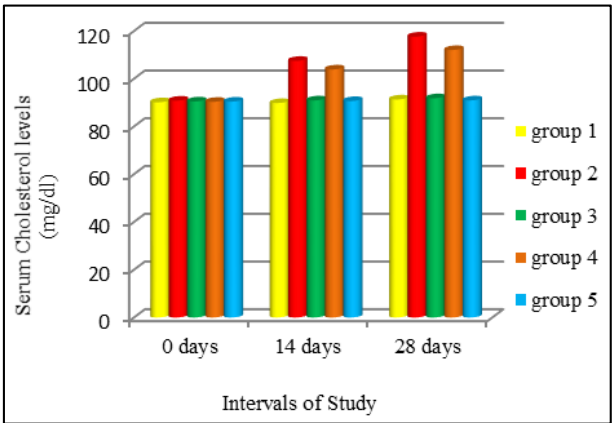
A


Serum AST levels (IU/L)
0 10 20 30 40 50 60 70 80 90 100
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

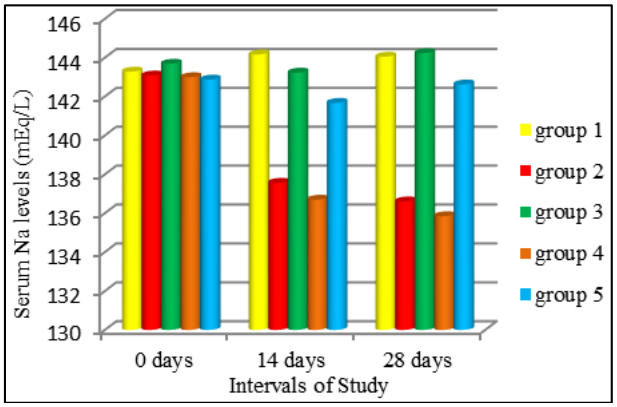
E


Serum glucose levels (g/dl)
130 135 140 145 150 155 160 165
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

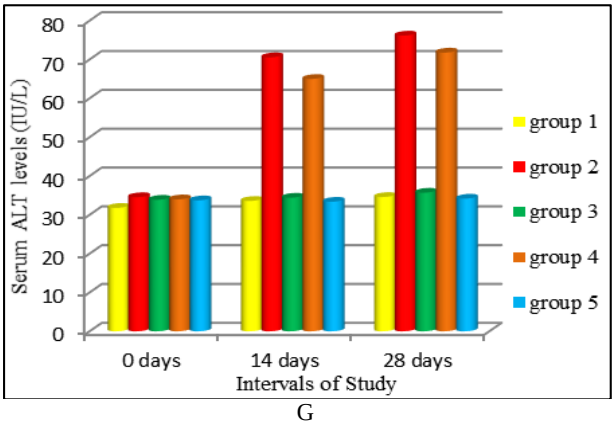
B


Serum Cholesterol levels (mg/dl)
0 20 40 60 80 100 120
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

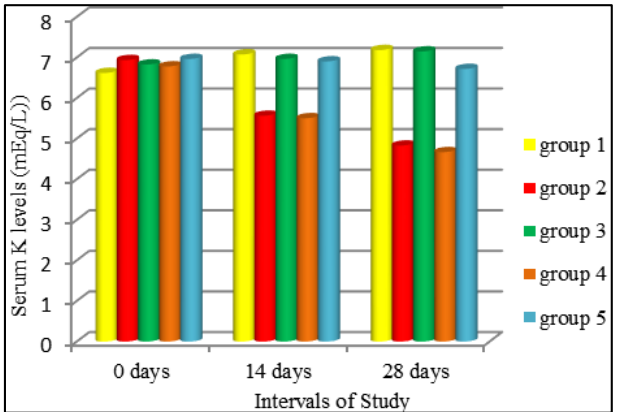
F


Serum Na levels (mEq/L)
130 132 134 136 138 140 142 144 146
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

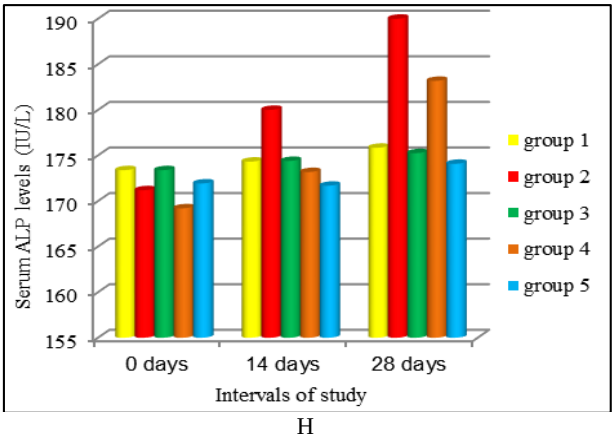
C


Serum ALT levels (IU/L)
0 10 20 30 40 50 60 70 80
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

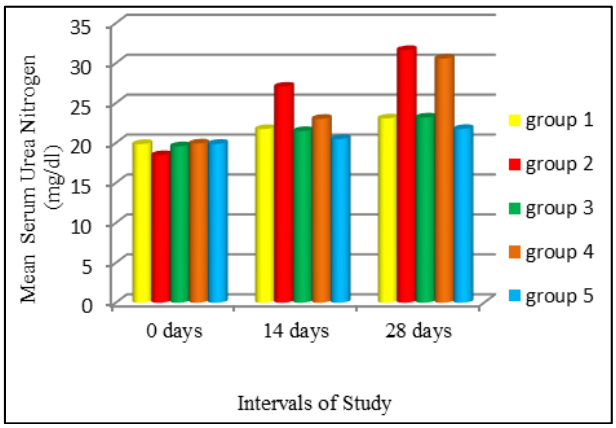
G


Serum K levels (mEq/L))
0 1 2 3 4 5 6 7 8
0 days
14 days
28 days
Intervals of Study
group 1
group 2
group 3
group 4
group 5

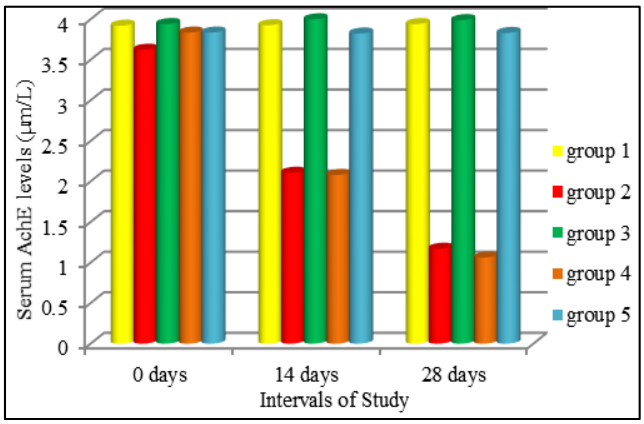
D


Serum ALP levels (IU/L)
155 160 165 170 175 180 185 190
0 days
14 days
28 days
Intervals of study
group 1
group 2
group 3
group 4
group 5

H



I



J

Fig 2: (a) Mean values of Serum Total Protein (g/dl) in experimental rats at different intervals of study. (b) Mean values of Serum glucose levels (g/dl) in experimental rats at different intervals of study. (c) Mean Serum Sodium levels (mEq/L) in experimental rats at different intervals of study. (d) Mean Serum Potassium levels (mEq/L) in experimental rats at different intervals of study. (e) Mean Serum Aspartate Transaminase levels (IU/L) in experimental rats at different intervals of study. (f) Mean Serum Alanine Transaminase levels (IU/L) in experimental rats at different intervals of study. (g) Mean Serum Cholesterol levels (mg/dl) in experimental male rats at different intervals of study. (h) Mean Serum Alkaline Phosphatase levels in experimental male rats at different intervals of study.(i) Mean Serum Urea Nitrogen (mg/dl) in experimental male rats at different intervals of study. (j) Mean Serum Acetyl choline Esterase levels in experimental male rats at different intervals of study.

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