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Ameliorative effect of *Tribulus terrestris* on haematological parameters against acephate induced subacute toxicity in Wistar rats

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Abstract
In the present research work ameliorative effect of *Tribulus terrestris* on subacute acephate induced toxicity was studied from general performance and evaluation of haematological parameters in male Wistar rats". Thirty male Wistar rats were divided equally into five groups, group I served as a control and treated with normal saline while group II treated with acephate @ 75 mg/kg bw p.o. once daily for 15 days and acephate was withdrawn for next 15 days. Group III treated with acephate @ 75 mg/kg bw for 30 days, group IV treated initial 15 days with acephate @ 75 mg/kg bw and *Tribulus terrestris* @ 100 mg/kg bw for rest of 15 days p.o. once daily and group V treated with acephate @ 75 mg/kg bw + *Tribulus terrestris* @ 100 mg/kg bw from day 1-30. From the results of study significant reduction ($P \leq 0.05$) was observed in mean body weight of rats from group II and III treated with acephate. However, combination groups (acephate + *Tribulus terrestris*) IV and V showed least decrease in mean body weight as compared to acephate alone treated groups. Rats treated with acephate alone showed significant ($P \leq 0.05$) decrease in Hb, PCV, TEC and TLC values. *Tribulus terrestris* treated groups IV and V showed least change in Hb, PCV, TEC and TLC values. In differential leucocytes count, the mean neutrophil count was found significantly ($P \leq 0.05$) higher in group II and III as compared to control. However, group IV and V showed least alteration in neutrophil count. The mean lymphocyte and monocyte count in group II and group III treated rats decreased significantly ($P \leq 0.05$) as compared to control however, group IV and V rats indicated non-significant differences as comparable to control group. In conclusion, acephate produces signs of deleterious effect on haematological parameters which were ameliorated by treatment with hydroethanolic fruit extract of *Tribulus terrestris*.

Keywords: acephate, *tribulus terrestris*, haematological, pesticide, wistar rats

Introduction
Practices of indiscriminate use of pesticides in agricultural field augmented among farmers, which results into detrimental effect on human and animal health. Misuse of highly toxic pesticides, coupled with a weak or a totally absent legislative framework in the use of pesticides, is one of the major reason for the high incidence of pesticides poisoning and hazards in developing countries [1]. Organophosphate insecticide (OP's) constitutes bulk of various insecticide which are used in veterinary, agriculture and public health practices. They are highly lipid soluble and act as direct or indirect acetylcholinesterase (AChE) inhibitors. Besides their acute toxic effects, OPs impose reproductive toxicity, impaired immune function and inflict various disorders and disease like cancer [2]. Acephate is an organophosphate insecticide used on citrus trees, food crops for treatment of seeds, on golf courses and in commercial as well as institutional services for insects control. Acephate was earlier assessed for its potential to produce reproductive toxicity in male mice after oral administration particularly decreased sperm count and motility [3]. Apart from various toxicity produced by acephate, it is also adversely affects the haematological parameters at subacute doses in rats [4]. The healing power of plants is accepted and recognized by common man and still they are mainstay of therapy in many parts of world. Among these medicinal plants *Tribulus terrestris* (*TT*) belong to the family Zygophyllaceae. Entire plant particularly fruits were widely used in Indian and Chinese medicine for treatment of many diseases. In folk medicine *TT* act as astringent, tonic, palliative, astringent, diuretic, lithotriptic, aphrodisiac and urinary disinfectant [5]. In ayurvedic medicine it is important constituent of Gokshuradi Guggul, which is used to support proper functioning of genito-urinary tract and also used to remove the kidney stones and since centuries traditional practitioner using it for treating sexual debility,

impotence and venereal diseases [5]. Therefore the present study is planned to investigate the effect of *Tribulus terrestris* on haematological alterations induced by acephate subacute doses.

Materials and Methods

Animals

Thirty male rats of 9-10 weeks of age were procured from National Institute of Bioscience, Pune. Before to start experiment all experimental animals were acclimatized for a week to the new environment under identical managemental and hygienic condition with ad-lib feed and water in laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS, Akola. The experimental protocol on laboratory animals was approved from Institutional Animal Ethics Committee (IAEC Reg. No. 312/CPCSEA) which follows the recommendations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Publication in 2010), New Delhi, India.

Test chemical

The technical grade acephate procured from Krishi Rasayan Export Pvt. Ltd., Samba, Jammu and used for inducing toxicity with batch no. S/KRE/L/T-485, Mfg. date- Dec.2018, Exp. date-Dec. 2020.

Hydro-alcoholic fruit extract

The fruit of *Tribulus terrestris* collected from local area and identified from expert taxonomist. The fruit powder 100g was immersed in 1000mL hydro-alcoholic solution with 60% ethanol and 40% distilled water in a conical flask stoppered with cotton plug. The conical flask was then kept in the orbital shaker for 72 hrs. At 150 rpm. Then extract was first filtered through muslin cloth and then through Whatmann filter paper no.1. The filtrate so obtained was collected in the large petri dish allowed to evaporate to obtain semisolid mass of extract. The extract so obtain was kept in desiccator for further use. The required concentration of extract used in study was prepared in normal saline.

Table 1: The details of experimental protocol was as under

Sr. No	Group	Treatment	No of animals
1	Group 1	Normal Saline	6
2	Group 2	Acephate @75mg/kg body wt./day p.o. for 15 days, then 15 days withdrawal period was given & animal were sacrificed on day 30.	6
3	Group 3	Acephate@ 75mg/kg body wt./day p.o. for 30 days	6
4	Group 4	Acephate@ 75mg/kg body wt./day p.o. for 15 days, then acephate was withdrawn and treated with *Tribulus terrestris* fruit extract @ 100 mg/kg body wt. /day for next 15 days, that is from day 16-30	6
5	Group 5	Acephate @75mg/kg body wt./day + *Tribulus terrestris* fruit extract@ 100 mg/kg body wt./day p.o. from day 1-30	6

Parameters studied

Clinical signs

During experimental period rats were thoroughly examined for clinical signs like agility, muscle tremors, diarrhoea, ataxia after acephate dosing.

Body weight

The body weights of rats were taken at weekly interval.

Haematological

For hematological estimation blood sample from six rats of each group were collected from the inner canthus of the rats at the end of experiment. Hematological parameters viz. Hb, PCV, TEC, TLC, MCV, MCH, MCHC were estimated as per the standard methods [6] using hemato-autoanalyser.

Statistical analysis

The data obtained during present investigations was analyzed by applying Completely Randomized Design (CRD) as described by Snedecor and Cochran [7].

Result and Discussion

The percent extractability of *Tribulus terrestris* fruit was found to be 7.4%. Initially experimental animals from the group II and group III rats treated with acephate @ 75 mg/kg b.wt for 15 and 30 days, respectively, did not exhibit any sign of toxicity but they exhibit agility immediately after administration of acephate which diminished after 15-20 min. The rats from group IV and V exposed to acephate also showed sign of agility like noted in group II. Second week onward animal from group II and III exhibit dullness, salivation, lacrimation, cyanotic tongue, watery and foul smell diarrhoea till the end of experiment. However, in group IV and V some of symptoms like dullness, depression, salivation and watery feaces as above were noted with mild intensity. Clinical observations in the present study like dullness, depression, watery feaces found in rats were also reported by different researchers [8, 9, 10] in acephate intoxicated rats.

At the end of 2nd week group II and III animals administered with acephate demonstrate decline in body weight indicating deleterious effects of acephate on body weight. However, comparatively lesser decrease in body weights values were found in group IV and V indicating ameliorative effect of *Tribulus terrestris* on body weight. At the end of 3rd and 4th week mean body weights were significantly ($P \leq 0.05$) decreased in group II and III compared to control, indicating more deleterious effect of acephate as compare to control. Group III rats showed drastic decrease in body weight as compared to control. In acephate+ *TT* combination treated groups rat comparatively less decrease in body weight was observed. In group V rats least decrease in body weight was observed as compare to control indicating ameliorative effect of *TT* on general performance of rats (Table 1).

Our findings of reduction in the body weight after exposure to acephate from second week onwards are in accordance with reports of Mishra [10]. According to Mishra [10] decreased body weight found due to displacement of nutrients in the feed by acephate results into primary malnutrition. Decreased body weight may be due to reduction of appetite and absorption of nutrients as insecticides produced harmful effect on gastrointestinal tract [11, 12].

Table 2: Weekly body weight (g) in control and different treatment groups of Wistar rats (n =6)

Group	0 week	1 week	2 week	3 week	4 week
I	155.833 ±2.38	165.00 ±2.24	178.33±1.66^{a}	184.16 ±1.53^{a}	185.83 ±1.53^{a}
II	157.83±0.94	170.00±1.29	161.66±1.054^{c}	155.00 ±1.82^{c}	150.00 ±1.05^{d}
III	158.00 ±2.44	166.66 ±2.10	150.00±1.29^{d}	150.00±1.82^{c}	140.83±1.53^{e}
IV	155.00 ±1.82	166.66 ±2.10	170.00±2.58^{b}	170.00 ±2.58^{b}	170.33 ±2.10^{c}
V	155.83 ±2.38	168.33 ±2.47	171.66 ±2.47^{b}	171.66 ±2.10^{b}	179.16 ±2.00^{b}
Significance/ N.S	N.S	N.S	*	*	*
* CD(0.05)	-	-	5.048	5.847	5.55

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels *P≤ 0.05, NS= Non-significant

The result on haematological parameters indicated that there was decrease in the concentration of hemoglobin in group II and group III rats treated with acephate. While in group IV and V haemoglobin concentration was showed least change as compare to control and acephate treated groups, indicating there is moderate restoration of hemoglobin values due to administration *Tribulus terrestris* in rats. In group II rats, Hb values on 15th day and on 15th day post withdrawal of acephate showed non-significant difference indicating no restoration in hemoglobin concentration even if acephate was withdrawn. The reason for decrease Hb due to disruptive action of insecticides on erythropoetic tissue responsible for lysis of the RBC [13] or hemolysis due to insecticides results into the impairment in process of hemoglobin synthesis [14].

There was significant (P≤0.05) decrease in PCV in group II and III treated with acephate as compare to control group indicating acephate produced alterations in PCV. At the end of experiment group IV and V showed significant improvement in values of PCV in comparison with group II and III indicating restoration in values of PCV in *TT* treated groups. The decrease in the value of PCV may be due to severe hemorrhages which cause dilution of blood produced by influx of cells and fluid from body stores [15].

There was significantly (P≤0.05) lower total erythrocyte count observed in group II and group III while erythrocyte counts in group IV and V were non-significantly differs from control group and found in the normal range indicating *Tribulus terrestris* delivers significant protective role against decrease TLC values due to treatment with acephate. Present findings of decreased TEC values in rats during acephate toxicity are in accordance with reports of Sharma *et al.* [4] in acephate subacute toxicity rats. Inhibition of erythrocytes may be due to disruptive action of insecticide on erythropoetic tissue [13].

The result indicated that though there are no significant significantly (P≤0.05) as compared to control. In group II TLC values at 30th day showed modest recovery after withdrawal of acephate. However, in *TT* and acephate combination groups IV and in group V highly significant (P≤0.05) recovery in TLC values has been observed as compared to control and acephate alone treated group. In group V remarkable recovery observed in TLC values and it was found to be comparable to control groups. The increased TLC in acephate intoxicated group is also reported by Farag *et al.* [16] and Sharma *et al.* [4].

Table 3: Haematological values related to erythrocytes in different groups at the end of experiment (n=6)

Group	Hb (g/dL)	PCV (%)	TEC (10^6/cu mm)	MCV (fL)	MCH (Pg)	MCHC (%)
I	12.87± 0.68^{a}	36.33± 1.40^{a}	6.98±0.14^{a}	52.18±2.54	18.52±1.26	35.39±1.51
II (15th day)	9.15±0.19^{c}	25.33± 1.33^{c}	5.45±0.22^{b}	46.94±3.21	17.68±0.78	38.57±3.07
II (30th day)	9.2 ±0.20^{c}	27.33± 0.98^{c}	5.61±0.26^{b}	48.97±1.99	15.99±1.03	32.85±2.33
III	8.1 ±0.07^{d}	21.33±0.66^{d}	4.43±0.13^{c}	48.25±1.71	18.34 ±0.54	38.15 ±1.22
IV	11.28±0.16^{b}	30.66±0.84^{b}	6.55±0.16^{a}	46.95±1.70	17.29 ±0.62	36.88±0.78
V	11.76±0.20^{b}	34.00±1.03^{a}	6.50±0.16^{a}	52.49±2.26	18.19 ±0.74	34.74 ±1.11
Significance/ NS	*	*	*	NS	NS	NS
* CD(0.05)	1.051	3.109	0.543	-	-	-

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels *P≤ 0.05, NS= Non-significant.

In present study significant increase in TLC was observed in acephate treated groups which could be due to activation of animal's defense mechanism as several compounds like inscecticides generate auto antibodies due to their interference with immune system [16] or due to stimulated lymphopoiesis and or enhance released of lymphocytes from lymph myeloid tissue [17].

Table 3: Hematological values related to differential leucocytes in different groups at the end of experiment (n =6)

Group	TLC (10^3/cu mm)	Neutrophil	Lymphocytes	Monocytes	Eosinophils	Basophils
I	6.38 ±0.25^{f}	16.5 ±1.74^{d}	75.83 ±1.35^{ab}	4.16 ±0.30^{a}	2.16 ±0.30	1.33 ±0.33
II (15th day)	16.21 ±0.27^{b}	23.66 ±0.42^{b}	71.16 ±0.47^{c}	2.33 ±0.21^{b}	1.16 ±0.40	1.33 ±0.33
II (30th day)	14.76 ±0.25^{c}	24.83 ±0.70^{ab}	71.00 ±0.81^{c}	2.00 ±0.44^{b}	1.50 ±0.22	0.83±0.30
III	17.88 ±0.42^{a}	27.66 ±1.17^{a}	66.33 ±1.40^{d}	2.16 ±0.47^{ab}	1.66 ±0.42	1.16 ±0.30
IV	11.80 ±0.45^{d}	16.83 ±1.75^{cd}	78.66 ±2.09^{a}	3.00 ±0.44^{ab}	1.50 ±0.22	1.00 ±0.25
V	7.92 ±0.26^{e}	20.16 ±0.74^{c}	72.50 ±1.33^{bc}	3.83 ±0.47^{a}	1.83 ±0.47	1.00 ±0.36
Significance/ NS	*	*	*	*	NS	NS
* CD (0.05)	0.963	3.488	3.885	1.176	-	-

Values indicate mean ± S.E. Mean values with common alphabet as superscript do not differ significantly. Significance levels *P≤ 0.05, NS= Non-significant

Differential leucocyte count (DLC)

Significantly higher (P≤0.05) number of neutrophils were found in group II and III as compared to control group. While the neutrophil count in group IV and V showed least alterations as compared to others treatment groups and control. In group II though acephate was withdrawn no reversal in the neutrophil count was observed. Lymphocyte count in group II and group III treated rats decreased significantly (P≤0.05) as compared to control. Lymphocyte count in group IV treated and in group V treated rats indicated non-significant differences and values are comparable to control group.

Similar finding of numerically increase in neutrophil count and decreased lymphocyte count is in line with the report of Mishra [10]. Observed significantly increased neutrophil percent in acephate intoxicated rats. Significant decrease in the total leucocytes count may be due to immunotoxic potential of acephate leads to suppression of leucopoiesis hence there is reduction in the lymphocytes count [9].

Group II and group III rats showed significant (P≤0.05) decrease in the monocyte count compared to control group, whereas group IV and V showed non-significant differences and restoration in the values as compared to control and acephate treated groups.

At the end of experiment eosinophil and basophil count found to be unaffected in different treatment and control groups (Table 3). Similar finding of unaffected mean values of eosinophil and basophils count upon exposure to acephate are in accordance with the findings of Mishra [10] observed non-significant difference in basophil count in acephate intoxicated rats.

Conflict of interest statement

The authors declare that they have no conflict of interest. Further it is stated that the manuscript is read and approved by all authors.

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