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Subacute oral toxicity of Dimethoate and ameliorative effects of *Quisqualis indica* in female Wistar rats

Kachave PB, Moregaonkar SD, Gangane GR, Bawanthade UR and Chavan RS

Abstract
In present study Dimethoate toxicity was induced in wistar rats to study haematological alterations in 24 Female wistar rats, were divided into four groups each comprised of six female rats. Group I as healthy control, Group II- Dimethoate (31 mg/kg b. wt) orally by gavage, Group III given Dimethoate @ 31 mg/kg b. wt + *Quisqualis indica* leaves powder 1% of feed daily and rats in group IV considered as plant control were fed with *Quisqualis indica* leaves powder 1% feed daily for 28 days.
Clinical signs such as Huddling, piloerection, diarrhoea, conjunctivitis, ataxia, staggering gait, spasm, respiratory distress, and dyspnoea were observed. Haematology revealed significant decrease in mean values of Hb, PCV, TEC, lymphocyte and significant increase in mean values of TLC in rats of treatment group. Administration of Dimethoate @ 31 mg/kg body weight through oral gavage daily for 28 days induced toxicity in female wistar rats and which was found ameliorated by daily feeding of *Quisqualis indica* leaves powder when fed @ 1% of feed.

Keywords: Dimethoate, Haematology, Rat

Introduction
India is a country with exceptionally high agricultural productivity, pesticides have been used in agriculture to increase food production by eliminating unwanted insects and controlling disease vectors. The use of pesticides causes severe environmental and health problem to humans. Pesticides are gradually absorbed by people through milk, meat, eggs, other animal products, as well as through fodder, water, air, and other feed items in animals. Pesticide residues are seen invarious animals. Among common pesticides, organophosphorus (OP) compounds are the most commonly used in agriculture today (El-Demerdash, 2011) [12].
At present India is the largest producer of pesticides in Asia and ranks 12th in world for use of pesticides with annual production of 90000 tons (Chitra *et al.*, 2006) [8]. About 20% of Indian food products contain pesticide residues above tolerance levels as (Mishra, 2014) [24]. Each year, approximately three million people are poisoned and 200,000 die as a result of pesticide poisoning worldwide, with the majority of them coming from developing countries. Dimethoate, one of the most important OP pesticides, is widely used in agriculture as a system and contact pesticide against a wide variety of insects and mites. It is also used to control houseflies indoors.
Antioxidants are essential in preventing cell damage caused by reactive oxygen species. According to recent research, antioxidants derived from plants that have free radical scavenging properties may be extremely beneficial in free radical-mediated diseases. Many synthetic antioxidant compounds have been shown to be toxic or potentially mutagenic, whereas plant-based medicines have fewer side effects in some cases than synthetic medications (Tapsell *et al.*, 2006) [42]. *Quisqualis indica* is also known as Rangoon Creeper. It is a Combretaceae family member and makes an excellent vine for outdoor gardens. Its seed and leaves are used medicinally, including as an antigelmintozone tool, particularly against tapeworm, and as a narcotic.

Materials and Methods
An experimental trial was carried out to study subacute oral dimethoate toxicity & ameliorative effects by *Quisqualis indica* on 24 female Wistar rats for a period of 28 days. The experimental study was evaluated through body weight, behavioral changes, hematological studies. At scheduled intervals *i.e.*, 0, 7th, 14th, 28th day the blood was collected. Twenty-four female Wistar rats were used for the present experimental study. All the rats were kept at Lab

animal facility available at College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Science University, Parbhani.
Identification marking was given to specific body part of each rat *i.e*., on head, back, tail, foreleg, hindleg with the help of picric acid solution and last rat used as unmark. Each cage was labeled with group number and the other detail information about respective group.

Collection of *Quisqualis Indica*
Quisqualis indica leaves were collected from nearby area of Parbhani, Maharashtra.

Preparation of plant Powder
Collected leaves were washed, air dried and then it was grinded by using electric grinder and powder of plant obtained and used for present study.

Details of Ex-Periment
The 24 Wistar rats were divided into 4 different groups, each group comprised of 06 female Wistar rats. Group I kept as healthy control with Normal feeding and watering. Group II kept as toxicity control group in which rats were gavaged with Dimethoate @ 31mg/kg of body wt. Group III used as treatment group Dimethoate @ 31 mg/ kg of body wt + *Quisqualis indica* leaves powder 1% of feed. Group IV used as plant control *Quisqualis indica* powder 1% of feed daily for 28 days.
During collection of blood for hematological and biochemical estimations rats were anesthetized by using diethyl ether on 0 day, 14th and 28th days of experiment.
Dimethoate procured from local market Parbhani.

Collection of Blood
Blood collection was done from retro-orbital plexus (Lateral canthus) with the help of capillary tube in EDTA vials for hematological study on 0-day, 14th and 28th day of experiment trial.

Result and Discussion
Clinical Signs/Behavioral Changes
During period of acclimatization, all experimental female rats appeared active alert and healthy with untoward manifestations suggesting proper acclimation. Also, on zero day of experiment they were normal, active and apparently healthy. From the 1st to the 28th day of the trial, all of the experimental female rats were carefully examined twice a day. Throughout the research period, there was no single death of experimental female rats.
All experimental animals from group I (Healthy control) of this group were normal, active and apparently healthy throughout the study period. Female rats in group II, shown there was reduction in water and food intake, dullness, depression and hair loss. Few minutes after dosing, dimethoate-treated rats exhibited varying degree of clinical symptoms like huddling, anxiety, slight tremor, piloerection, diarrhea, increased lacrimation and conjunctivitis. Additional behavioral changes like convulsions, muscular twitching, tremors, ataxia, staggering gait, respiratory distress and dyspnea observed. Similar observations were recorded by Sanderson & Edson, (1964) [33], Farag *et al.*, (2006) [14], Saafi *et al*., (2011) [27], Amara *et al.*, (2012) [2], Salama (2012) [31] and Holy *et al*., (2015) [17] after OP insecticide toxicity in rats.
The clinical signs in group II experimental female rats were consistent with cholinergic symptoms associated with cholinesterase inhibition which is the principal mode of action of organophosphorus compounds (Sarkar *et al.*, 2003 and Sharma *et al*., 2005).
Clinical manifestations of OP insecticide poisoning are caused by excessive synaptic accumulation of acetylcholine (Senanayake and Johnson, 1982). OP compounds irreversibly inhibit the enzyme acetylcholinesterase resulting in excessive accumulation of acetylcholine, leading to the paralysis of cholinergic transmission in the central nervous system, autonomic ganglia, parasympathetic nerve endings, some sympathetic nerve endings and neuromuscular junction (Viswanathan and Srinivasan, 1964).
Clinical signs observed in female rats of group III during the first two weeks of the period of the experiment were similar to the behavioral changes observed in rats of group II. The severity of symptoms in group III was considerably lower than group II. Rats were slightly lethargic and there was piloerection and increased urine output.
The clinical manifestations showed by female rats in this group indicated that there were no much ameliorative effects of feeding *Q. indica* leaves powder on altered behavioral changes caused by dimethoate toxicity. But reduced intensity of clinical signs might be because of *Quisqualis indica* which has antioxidant, hepatoprotective, nephroprotective, antidiabetic, anti-inflammatory, antidiarrheal, and antiulcer effects. The reports of Bose *et al*., (2009), Sahu *et al.*, (2012) [30], Yadav *et al.*, (2011), and Kumar *et al.*, (2020) supports the findings observed in present study.
The female Wister rats in Group IV were active and apparently healthy as in control group I throughout experimental period.

Average Body Weight: Body weights of experimental rats were recorded at weekly intervals for noting average body weight throughout the study period. Table 1, illustrate the average weekly body weight of experimental female rats from the first to fourth week of the research.

Table 1: Mean Group value of Average body weight (gm/week) in Female

P of rats		Average body weight of Female rats (gm/week)				
		Intervals of study				
		0 Day	7th Day	14th Day	21st Day	28th Day
I		181.66 ± 7.49	187.41 ± 7.82	190.66 ± 9.02	193. 66^{a} ± 7.96	197.0^{a} ± 13.89
II		185.66 ± 8.02	178.66 ± 7.45	169.50 ± 5.02	150.33^{b} ± 9.02	146.83^{b} ± 7.00
III		183.33 ± 9.69	181.73 ± 8.70	178 ± 9.8	175.16^{a} ± 9.7	179.66^{a} ± 7.03
IV		181.52 ± 8.34	186.16 ± 8.96	190.45 ± 9.76	193.83^{a} ± 7.90	195.0^{a} ± 8.80
CD Values	@1%	-	-	-	31.95	32.48
	@5%	-	-	-	23.42	23.82
Statistics		NS	NS	NS	HS	HS

Means bearing similar superscripts in column and rows do not differ significantly (P< 0.01), (P< 0.05)

On 0, 7th, 14th, 21st and 28th day, mean values of body weights of female rats in all four group were 181.66 ± 7.49, 187.41 ± 7.82, 190.66 ± 9.02, 193.66 ± 7.96 and 197.0 ± 13.89, respectively. However, the values were significantly high at 21st and 28th day of study as compared to other groups. It can be concluded that, there was increasing trend of mean values of body weights at higher intervals of study On evaluation of data of this group on 0, 7th, 14th day body weights were 185.66 ± 8.02, 178.66 ± 7.45 and 169.50 ± 5.02 respectively which remained that statistically non-significant but numerically declining at this interval of study. Body weight recorded on the 21st and 28th days of the study period, showed that there was a significant decrease in mean body weights of female rats in group II.

At 21st and 28th day of study period, these values were 150.33± 9.02 and 146.83 ± 7.00. When compared to the respective control group 193.6 ± 7.96 and 197.0 ± 13.89 at that interval of study. Values were mean decreasing trend in respect of mean body weights at higher intervals of study.

Similar findings of significant reduction in body weight of female Wistar rats as observed in present trial goes well with earlier reports of Hassan *et al.*, (1994), Chung *et al.* (2002) [9], Sharma *et al.*, (2005), Sunmonu and Oloyede (2010) [39], Farag *et al.*, (2006) [14], Heikal *et al.* (2011), Saafi *et al.*, (2011) [27], Amara *et al.*, (2012) [2], and Lone *et al.* (2017) who studied body weight in OP toxicity during their studies.

Reasons for weight loss might be due to protein declination which could be due to variation in electrolyte balance leading to water loss in tissue. The reduced intake of feed attributed to protein catabolism, which might contribute to observed kidney injury.

Also, reduction in mean body weight in female rats of group III at 21st and 28th day were 175.16 ± 9.7 and 179.66± 7.03 respectively as compared to respective control group (193. 66 ± 7.96 and 197.0 ± 13.89) but, these values were increased as compared to group II. There was a significant change in mean female body weights on the 21st, and 28th days of the study period. The improvement in body weight gain in this group might due to ameliorative effects of *Quisqualis indica.*

The mean values of body weight on 0, 7th, 14th, 21st and 28th day were 181.52 ± 8.34, 186.16 ± 8.96, 190.45 ± 9.76, 193.83 ± 7.90 and 195.0 ± 8.80, respectively. These mean values were having increasing trend at higher intervals of study. Similarly, average body weights of rats in group IV were statistically comparable with mean values of group I (control) and group III.

Hematological Studies

Haemoglobin Concentration (gm/dl)

The mean (±SE**)** values of hemoglobin (gm/dl) at different intervals of the study period in different groups of female rats are shown in table No. 2

Table 2: Mean values of Haemoglobin (Hb, gm/dl) in female wistar rats

Group of rats		Haemoglobin (Mean ± S.E. gm/dl)		
		Intervals of study		
		O Day	14th Day	28th Day
I		13.60± 0.31	13.93^{a}± 0.22	13.81^{a}± 0.43
II		14.00 ± 0.28	12.21^{b}± 0.19	10.91^{b} ± 0.35
III		13.95 ± 0.57	12.98^{ab}± 0.33	12.91^{a} ± 0.33
IV		13.71 ± 0.40	13.78^{a}± 0.10	13.80^{a} ± 0.23
CD Value	@ 1%	-	-	1.475
	@ 5%	-	1.279	1.080
Statistics		NS	S	HS

Means bearing similar superscripts in column and rows do not differ significantly (P< 0.01), (P< 0.05)

At 0-day experiment There were no significant differences in the mean values of hemoglobin concentration in female rats from experimental groups I to IV, at the 0 day of the research period, and values were 13.60 ± 0.31, 14.00 ± 0.28, 13.95 ± 0.57, and 13.71 ± 0.40, respectively. On the 14th and 28th days of the experiment, the mean (±SE) hemoglobin values were 13.93 ± 0.22 and 13.81 ± 0.43, respectively. Hb values were within normal range and which represents the rat's healthy status over the course of the experiment.

Group II (dimethoate control) On 14th day of trial, the mean hemoglobin concentrations in female rats of groups II were 12.21 ± 0.19 gm/dl. At this interval of experiment, mean hemoglobin concentrations in group II rats was found decreased significantly (P< 0.01) as compared to mean Hb values in rats of groups I and IV.

At the 28th day, the mean hemoglobin concentrations in female rats from group II were 10.91 ± 0.35 respectively. The mean values of hemoglobin concentration in rats of group II were found significantly lower than group I, group III and group IV.

As the trial progressed, the mean value of hemoglobin concentration dropped significantly in DM toxicated rats as compared to the corresponding control group value. Similar observations of decrease in hemoglobin were reported by Kalender *et al* (2006) [21] in diazinon toxicity in rats, Ambali (2007) [3] in chlorpyriphos toxicity in mice, Jain (2009) [19] in malathion toxicity in rats. Similarly, Salih, (2010) [32], Dogan & Can, (2011), Khogali *et al*., (2014)**,** Poddar (2014) [7] and Holy *et al*., (2015) [17] recorded decrease Haemoglobin concentration in OP compound toxicated rats at different intervals in their respective studies.

It was discovered that OP insecticides have an effect on haematological parameters in humans (Patil *et al.,* 2002). Some haematological and biochemical parameters in experimental animals were altered by OP insecticides (Hassan *et al.*, 1988; Jacobsen *et al*., 2004 [18] and Kalender *et al.*, 2005) [20].

The decrease in hemoglobin along with the decrease in RBC production of might be due to the effect of pesticides on erythropoietic tissue in rats. Many steps in heme biosynthesis are inhibited by pesticide residues and this might be a possible pathophysiological reason for the inverse proportion of the results obtained.

Iron is obtained from stored ferrite as well as a from dietary source which is required for Hb production. Hb synthesis necessitates the use of iron, which is typically obtained from stored ferritin and dietary sources. Iron deficiency may be caused by intoxicated rats due to reduced general food intake

and a lack of supplemental iron supply. When the supply of iron is insufficient, the rate of hemoglobin synthesis decreases throughout all stages of erythrocyte maturation. Rats given sub-acute doses of other organophosphate insecticides showed a similar decrease in R.B.C. and hemoglobin concentration. One of the most important factors to be considered in reduction of RBC count is the production of the hormone erythropoietin (Morowati, 1998) [25].

Group III (dimethoate + *Q. indica* treatment) At 14th and 28th day of experiment The mean value of the hemoglobin concentration (g/dl) in female rats of this group at 14th and 28th day intervals of study were 12.98±0.33 and 12.91± 0.33. These were significantly improved than values in rats of group II and comparable to the values in rats of group I and IV. That improvement might be resulted due to antioxidant properties and hepatoprotective effect of the plant leaf powder.

Group IV (*Q. indica* control) At 14th day and 28th day of experiment Mean value of the hemoglobin concentration (g/dl) of this group at 14th and 28th day were 13.78± 0.10 and 13.80 ± 0.23 respectively. The mean values of hemoglobin in female rats of group IV did not differ significantly than the values of control group at 14th and 28th day interval of study, indicating no adverse effect of plant leaf powder feeding on Hb values of rats. Perusal of the literature did not reveal any report in support of this observation.

Packed Cell Volume (%)

The mean values (SE±) of PCV (%) in different groups of experimental rats at scheduled intervals are given in table 3.

Table 3: Mean Values of Packed Cell Volume (PCV %) in Female wistar rats

Group of rats		Packed Cell Volume (Mean ± S.E. %)		
		Intervals of study		
		O Day	14th Day	28th Day
I		39.76 ± 0.56	39.33^{a}± 0.42	39.16^{ab} ± 0.54
II		39.51 ± 0.37	35.75^{b} ± 0.65	34.75^{c} ± 0.83
III		38.60 ± 0.75	36.08^{b} ± 0.30	36.83^{bc} ± 1.24
IV		39.41 ± 0.61	40.50^{a} ± 0.61	40.16^{a} ± 0.60
CD value	@ 1%	-	3.308	3.432
	@ 5%	-	2.404	2.512
Statistics		NS	HS	HS

Means bearing similar superscripts in column and rows do not differ significantly ($P< 0.01$), ($P< 0.05$)

At 0-day experiment There were no significant differences in the mean values of PCV concentration in female rats from experimental groups I to IV, at the 0 day of the research period, and values were 39.76 ± 0.56, 39.51 ± 0.37, 38.60 ± 0.75 and 39.41 ± 0.61, respectively.

Group I (Healthy control) At 14th and 28th day of experiment Mean values of PCV in female rats of experimental group I at 14th and 28th day interval was 39.33 ± 0.42 and 39.16 ± 0.54, respectively and they lie within normal physiological limits.

Group II (Dimethoate control) At 14th day experiment Mean packed cell volume percentages of group II female rats were (35.75 ± 0.65) significantly ($P< 0.01$) lower than groups I and IV, but it was statistically comparable with mean values of PCV in rats of group III. At 28th day experiment Mean (±SE) packed cell volume percentages of group II rats were (34.75± 0.83) significantly ($P< 0.01$) lower than female rats of group I and IV. However, it was statistically comparable with mean values of group III but were numerically lower.

There was significant decline in mean values of PCV in rats' group II as compared to control group. The hematological changes were mainly in the hematocrit which seems to be due to malabsorption of nutrients or the hyperactivity of the animal. Group III (dimethoate + *Q. indica* treatment) At 14th day of experiment Mean values of PCV in female rats of experimental group III at 14th interval were 36.08 ± 0.30, were found to be significantly reduced than control group (39.33± 0.42) but statistically comparable with group II. At 28th day of experiment Mean values of PCV in female rats of experimental group III at 28th day interval (36.08 ± 0.30) was found to be significantly reduced than control group (39.16 ± 0.54). PCV values found to be improved than toxicity control group that might due ameliorative effects of *Q. indica*.

Group IV (*Q. indica* control) At 14th and 28th day of experiment. The mean value of the PCV (%) of this group at 14th and 28th day were 40.50 ± 0.61 and 40.16 ± 0.60 respectively. The mean values of PCV in female rats of group IV did not differ significantly than the values of control group at 14th and 28th day interval of study.

Total Erythrocyte Count (millions/cumm)

The mean (±SE) values of TEC in female rats of experimental groups at scheduled intervals are mentioned in table no. 4. At 0 day and 14th day of experiment, the mean values of TEC in female rats of groups I to IV found non-significant at this interval. But values of group II and III were reduced numerically than control groups on 14th day of experiment

Table 4: Mean values of Total Erythrocyte Count (TEC, millions/cumm)

Groups of rats		Total Erythrocyte Counts (Mean ± S.E. millions/cumm)		
		Intervals of study		
		O Day	14th Day	28th Day
I		7.05 ± 0.33	7.11 ± 0.07	7.11^{a} ± 0.11
II		7.20 ± 0.24	6.50 ± 0.18	6.36^{b}± 0.14
III		7.16 ± 0.22	6.80 ± 0.46	6.65^{ab}± 0.18
IV		6.91 ± 0.37	7.03 ± 0.13	7.10^{a}± 0.19
CD value	@1%	-	-	0.654
	@5%	-	-	0.479
Statistics		NS	NS	HS

Means bearing similar superscripts in column and rows do not differ significantly ($P< 0.01$), ($P< 0.05$)

Group I (healthy control) At 28th day of experiment The mean values of TEC in experimental female rats of group I were (7.11 ± 0.11 million /cumm, 28th day interval mean TEC values were found normal and within hence physiological limits. Group II (dimethoate control) At 14th and 28th day of study period, values of TEC in rats of Group II were 6.50 ± 0.18 and 6.36 ± 0.14, respectively. There was numerical and significant reduction in mean values of TEC in female rats of group II than the rats of control group at 28th day of assessment respectively.

A similar decrease in mean value of TEC was observed by Reena *et al*., (1989) [26] who studied dimethoate toxicity in rats. In addition, the findings in respect to TEC reported by Kalender *et al*., (2006) [21], Aambali *et al.*, (2007) [3], Jain *et al*., (2009) [19], El-Deeb *et al.*, (2007) [11], Salih (2010) [32], Dogan and Can (2011), Poddar and Jha (2014) [7], Khogali *et al*., (2005) [22] and Holy *et al.*, (2015) [17] are in concurrence with present results.

As noted in present trial, the decrease in the RBC might be due to the effect of pesticides on erythropoietic tissue in rats. According to Enan (1976), reduction in RBC count and hemoglobin concentration could be attributed to internal haemorrhages. One of the most important factors to be considered in reduction of RBC count is the production of the hormone erythropoietin (Morowati, 1998) [25]. Due to toxicity, there is destruction of erythrocytes or inhibition of erythrocytes production, which decreases the R.B. Cs in rats. Deficiency of vitamin B_{12} and folic acid leads to impaired synthesis of nucleic acid resulting in defective maturation of erythrocytes and their nuclei (Abdollahi M, *et al.*, 2004) [1]. These effects of organophosphorus pesticides could be due to their ability to form free radicals (Hazarika *et al*., 2003; and Vidyasagar *et al*., 2004) [15].

Group III (Dimethoate + *Q. indica* treatment) At 14th and 28th day of study interval, mean values of TEC in rats of group III were 6.80 ± 0.46 and 6.65 ±0.18, respectively. There was non-significant and significant reduction in mean values of TEC in females' rats of this group than the rats of control group respectively. However, in female rats of group III, the mean values of TEC were improved either compared to female rats of group II. The comparative reduction in TEC in female rats of group III than group I might due to toxicity resulting into destruction or inhibition of erythrocytes production. However, the improvement in mean TEC in female rats of group III than group II might be due to protective effect of plant.

The treatment of *Quisqualis indica plant* improved mean values of PCV, TEC and hemoglobin concentration in rats of group III than group II. It might have occurred due to antioxidant, hepatoprotective and nephroprotective properties possessed by plant leaf powder of *Quisqualis indica.*

Group IV (*Q. indica* control) At 14th day and 28th day of experimentThe mean (SE±) value of TEC in female rats group IV at 14th and 28th day were 7.03 ± 0.13 and 7.10± 0.19, respectively. The mean values of TEC in female rats of group IV did not differ significantly when comparison was made amongst treatment and control group at 14th and 28th day interval of study.

Total leukocyte count (thousand/cumm)

Table 5 shows mean (SE±) values of TLC in rats of different groups I to IV at different time intervals of experiment.

Table 5: Mean value of Total Leukocyte Count (thousand/cumm)in female rats

Group of rats		Total Leukocyte Count (Mean ± S.E. thousand/cumm)		
		Intervals of study		
		O Day	14th Day	28th Day
I		8.45± 0.47	8.81^{b}± 0.60	9.10^{bc}± 0.18
II		8.48± 0.19	11.83^{a}± 0.65	12.1^{a}± 0.74
III		8.55 ± 0.52	11.26^{a}± 0.88	11.33^{ab}± 0.54
IV		8.30± 0.90	8.66^{b} ± 0.82	8.69^{c} ± 0.03
CD value	@1%	-	-	-
	@5%	-	2.206	2.512
Statistics		NS	S	S

Means bearing similar superscripts in column and rows do not differ significantly ($P< 0.01$), ($P< 0.05$)

At 0 day of experiment The mean values of TLC in rats of experimental groups I to IV at 0 day were 8.45± 0.47, 8.48± 0.19, 8.55 ± 0.52 and 8.30± 0.90 respectively. These were found to be comparable and within physiological limits Group I (Healthy control) At 14th and 28th day of study, mean values of TLC in female rats of group I were thousand/cumm 8.81± 0.60 and 9.10 ± 0.18, respectively. The values were within physiological limits.

Group II- (Dimethoate control)

At 14th and 28th day of experiment As compared to respective control group, there was significant incline in mean values of TLC in rats of group II at 14th and 28th day interval respectively. While mean values of TLC in female rats of group II were comparable with group III. These values in female rats of group II remained comparable with group III but increased significantly than group I. This increase in leukocyte counts may indicate an activation of the animal's defense mechanism and immune system. It could also be due to the tissue damage and necrosis caused by the pesticide. Some OP insecticides may cause increase in WBC. Decrease in WBC was observed in monocrotophos-treated chickens (Garg *et al.*, 2004) [15]. In the present study dimethoate caused increase of WBC.

The leukocytosis observed in present study indicates an immune system to protect the rats against toxicity that might have been caused by chemical and also secondary infections, which may be contracted after the weakening condition of the rats. Leukocytosis, which may be directly proportional to the severity of the causative stress condition may be attributed to an increase in leukocyte mobilization.

Group III (dimethoate + *Q. indica* treatment) At 14th and 28th day of experiment The mean values of TLC at 14th and 28th day of experiment were 11.26 ± 0.88 and 11.33 ± 0.54 respectively. These values found to be increased significantly as compared to control group I and comparable with group II. The values of TLC of this group improved than toxicity control group that might due to anti-inflammatory effect

shown by *Quisqualis indica*, (Bhangale and Qureshi, 2021) [5]. Group IV (*Q. indica* control) At 14th and 28th day of experiment The mean values of TLC at 14th and 28th day of experiment were 8.66 ± 0.82 and 8.69c± 0.03, respectively. These values remained comparable to rats in group I.

Differential leukocyte count (DLC, %)

Mean (±SE) values of Differential leukocyte count (%) in rats at different intervals of study in different groups are depicted in table No. 6.

Group I (Healthy control group)

Table No 6: Neutrophil counts (%)

Mean (±SE) values of neutrophil counts in rats at different interval of study in different groups are depicted in table 6.

At day 0 of experiment, the mean (±SE) neutrophil count (%) values of experimental rats in group I to group IV were 26.66 ± 1.14, 27.16 ± 0.79, 27.83 ± 0.94 and 27.66 ± 1.33 respectively. At this stage of experiment, the mean neutrophil counts (%) were statistically non-significant at this interval of study. At 14th and 28th day of experiment. At this stage of study, the mean values of neutrophil counts (%) were 27.66 ± 1.14 and 27.16 ± 0.40 respectively in normal physiological range.

Group II (dimethoate control) At 14th and 28th day of experiment At this interval of experiment neutrophil count (%) were 30.16 ± 0.79 and 31.16 ± 0.47 respectively. These values were markedly increased as compared to healthy control group and plant control group. Grossly, the mean counts of neutrophil percentage of group II showed marked elevation throughout the experiment due to pesticide toxicity in rats. These findings are similar with Celik and Suzek (2008) [6], Jain *et al*., (2009) [19], Sunmonu and Oloyede (2010) [39] and Sahhaf *et al*., (2006) [29]. Garg *et al*., (2004) [15] noted neutophilia as characteristic feature of OP pesticide toxicity in rats. Present study noted the degenerative and necrotic changes in visceral organs and lymphoid tissue which might be reason of increased neutrophils as toxin mediated inflammatory response in toxicated rats.

Group III (dimethoate + *Q. indica* treatment)

At 14th and 28th day of experiment

At 14th and 28th day of experiment the mean values of neutrophil counts (%) were 27.62 ± 0.94 and 26.41 ± 0.49, respectively. The mean values at this interval of study are similar with normal control group. Neutrophil count in this group improved than toxicity group and might be due to anti-inflammatory effect of *Quisqualis indica*, (Bhangale and Qureshi, 2021) [5].

Group IV (*Q. indica* control) At 14th and 28th day of experiment

At 14th and 28th day of experiment the mean values of neutrophil counts (%) were 27.34 ± 1.33 and 27.42± 0.33, respectively mean values at this interval of study are similar with normal control group.

(b) Lymphocyte count (%)

Mean (±SE) values of lymphocyte count (%) in rat's different intervals of study in different groups are depicted in table 6 Group I (healthy control) At day 0 day of experiment the mean (±SE) lymphocyte count (%) in experimental rats in group I to group IV were 64.66± 1.50, 61.83 ± 0.79, 66.66 ± 0.88 and 66.16± 1.32, respectively. At this stage mean lymphocyte count (%) statistically did not differ significantly within experimental groups.

Table 6: Mean value of Differential leukocyte counts (%) in female rats

Group of rats		Interval of study		
		0 Day	14th Day	28th Day
		Neutrophil count (%)		
I		26.66 ± 1.14	27.66^{a} ± 1.14	27.16^{b} ± 0.40
II		27.16 ± 0.79	30.16^{b} ± 0.79	31.16^{a} ± 0.47
III		27.83 ± 0.94	27.62^{a} ± 0.94	26.41^{b} ± 0.49
IV		27.66± 1.33	27.34^{a} ± 1.33	27.42^{b}± 0.33
CD Value	@1%	-	-	1.736
	@5%	-	2.029	1.273
Statistics		NS	S	HS
Basophil count (%)				
I		1.66 ± 0.42	0.83 ± 0.21	1.16 ± 0.40
II		0.83 ± 0.49	0.33 ± 0.40	1.33 ± 0.42
III		0.50 ± 0.42	1.00 ± 0.47	1.50 ± 0.43
IV		0.75± 0.48	1.83 ± 0.36	1.00 ± 0.36
Statistics		NS	NS	NS
Eosiniphil count (%)				
I		2.33 ± 0.21	2.00 ± 0.30	2.16 ± 0.34
II		2.16 ± 0.16	2.33 ± 0.25	2.02± 0.16
III		2.66 ± 0.33	2.66± 0.21	2.55 ± 0.49
IV		2.83 ± 0.30	2.50 ± 0.22	2.34 ± 0.25
Statistics		NS	NS	NS
Lymphocyte values (%)				
I		64.66± 1.50	67.08 ± 0.62	67.08 ± 0.62
II		61.83 ± 0.79	66.83± 0.40	63.83^{b}± 0.92
III		66.66 ± 0.88	66.54 ± 0.27	67.11^{a}± 0.79
IV		66.16± 1.32	66.70 ± 0.68	67.86^{a}± 0.61
CD Value	@1%	-	-	2.112
	@5%	-	-	1.540
Statistics		NS	NS	HS
Monocyte count (%)				

I	2.83 ± 0.30	2.41± 0.76	3.16± 0.30
II	2.41 ± 0.16	2.33 ± 0.60	2.66± 0.55
III	2.63± 0.61	2.83 ± 0.65	2.50 ± 0.68
IV	2.16 ± 0.49	2.61± 0.63	2.33 ± 0.49
Statistics	NS	NS	NS

At day 14th and 28th day of experiment the mean (±SE) lymphocyte count (%) in experimental rats in this group were 67.08 ± 0.62 and 67.08 ± 0.62 at this stage mean lymphocyte count (%) were statistically did not differ significantly within experimental groups.

Group II (Dimethoate control) At day 14th nonsignificant and at 28th day significant reduction of experiment the mean (±SE) lymphocyte count (%) was noticed in group II. as compared to control group. Organophosphate toxicity was characterized by significant decrease in lymphocyte count (Garg *et al*., 2004) [15]. Group III (Dimethoate + *Q. indica* treatment) At 14th and 28th day of experiment the mean values of lymphocyte counts (%) were 66.54 ± 0.27 and 67.86 ± 0.61. The mean values of group III were similar to control group.

Group IV (*Q. indica* control) At 14th and 28th day of experiment the mean values of lymphocyte counts (%) were 66.70 ± 0.68 and 67.86 ± 0.61 less and similar with control group.It has been observed that there was non-significant variation in mean values of Basophil, Eosinophil and Monocyte in female rats of all experimental groups when counted at scheduled intervals of experimental trial.

Conclusions

1. Administration of Dimethoate @31 mg/kg body weight through oral gavage daily for 28 days induced toxicity, which was evidenced by haemato-biochemical alterations in female Wistar rats.
2. The feeding of *Quisqualis indica* leaves powder through feed daily for 28 days resulted into better amelioration of toxic effects induced by dimethoate in female Wistar rats.

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