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# INFLUENCE OF PARAQUAT (PQ) INDUCED ACUTE TOXICITY ON BODY WEIGHTS AND HAEMOTO-BIOCHEMICAL PARAMETERS IN EXPERIMENTAL RATS

Veterinary Science

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## ABSTRACT

Paraquat (PQ) is a highly toxic potent herbicide promoted by the United States for use in Mexico to destroy marijuana plants in the year 1955. There have been frequent PQ poisoning incidents which have become a severe public health issue all over world, especially in Asian region. The present experiment was designed to study the acute toxicity effects of PQ on body weights and haemato-biochemical alterations among 32 male *Wistar* rats (two groups) at different time intervals i.e. 24, 48, and 72 hrs. Group 1 served as control and group 2 rats were injected with PQ at the rate of 24 mg/kg body weight intraperitoneally as a single dose. Average body weight gains were recorded during 24, 48 and 72 hrs of experiment. A day before sacrifice the blood and serum samples were collected from 6 rats in each group for TEC, Hb and platelets, ALT, AST and creatinine respectively. The obtained data was statistically analyzed by using SPSS software.

## KEYWORDS:

Paraquat, ALT, AST,TEC, TLC, Hb, platelets and creatinine.

## INTRODUCTION

The pesticide is defined as any substance or a mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. The continuous and erroneous usage of pesticides has resulted in their widespread distribution into the environment, which are toxic not only to insects and pests but also to animals and human beings at different bioavailability levels (Mondal *et al.,* 2012).

Globally the pesticides are categorized into four main groups *viz.,* are herbicides, fungicides, insecticides and rodenticides. Among all, herbicides PQ is positioned pinnacle in global agrochemical business. All herbicides are phytotoxic chemicals used for destroying various weeds and inhibiting their growth. According to EPA (2011), in 2007 world pesticide expenditures was around $ 39.4 billion in which herbicides accounts 40% followed by insecticides, fungicides and others.

The paraquat (1, 1′-dimethyl-4, 4′-bipyridilium dichloride - PQ), is one of the most widely used herbicides and holds a largest global share of the herbicide market till today. It is a non-selective quaternary nitrogen herbicide, is commonly used as a desiccant and defoliant in a variety of crops all around the world (Dasta, 1978; Bismuth *et al.,* 1982 &1990 and Raghu *et al.,* 2013). For the past 60 years PQ is considered as most toxic compound, which is moderately hazardous herbicide and placed in class II poison by WHO (2009) due to its acute toxicity. Besides the WHO opinion, the PQ is highly toxic towards animals (Cope, 2004 and June *et al.,* 2016) and humans with fatalities were reported by Kelly *et al.,* (1978) and Florkowski *et al.,* (1992). The main risks are due to deliberate dose dependent ingestion results in multiple organ failure and death (Florkowski *et al.,* 1992). Other routes of toxic exposure of PQ are inhalation, ocular and skin (Bataller *et al.,* 2000 and Baharuddin *et al.,* 2011). Among all skin exposure is more common and causes irritation on prolonged contact leads to severe systemic toxicity or even death (Bataller *et al.,* 2000 and Marrs & Adjei 2003).

## MATERIALS AND METHODS

In the present study a total of 36 male *Albino Wistar* rats weighing between 180-240 g were procured from G. Pullareddy College of Pharmacy, Hyderabad. The rats were housed in solid bottom polypropylene cages at Ruska Labs and were maintained under controlled environment (Temperature 20-22$^0$C) throughout the course of experiment. Sterile rice husk was used as standard bedding material. All the rats were provided with standard pellet diet and deionized water *ad libitum* during experimental period. All the experimental animals were closely observed thrice daily for clinical signs and mortality, if any. The experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (IAEC-GPRCP/IAEC/07/17/01/PCL/AE-3-Rats-M-12). Paraquat was procured from a wholesale pesticide outlet in Hyderabad under the trade name Milquat (24% W/V solution).

**The experimental design adopted for the present study is shown in Table 1.**

| Group | No of Animals | Treatment |
|---|---|---|
| Group-I | 18 | Basel diet |
| Group-II | 18 | Paraquat (I/P) single dose @24 mg/kbw |
| Group-II(a) | 06 | Paraquat (I/P) single dose @ 24 mg/kbw |
| Group-II(b) | 06 | Paraquat (I/P) single dose @ 24 mg/kbw |
| Group-II(c) | 06 | Paraquat (I/P) single dose @ 24 mg/kbw |

Prior to blood collection, the selected experimental rats were starved for 12 hours. Six rats from each group were used for blood collection (Approximately 2-3 mL, through retro-orbital plexus through capillary tube) during 24$^{th}$, 48$^{th}$ and 72 hours intervals of experiment into an anticoagulant coated vaccutainers {(K3- EDTA tube, 13mm x 75mm, 4mL (Rapid Diagnostics Pvt, Ltd., Delhi)} to carry out all hematological parameters and also into a clot promoting {(Vit K-coated-clot activator tube-plain 13mmx 75mm, 5mL) (Rapid Diagnostics Pvt. Ltd., Delhi)} vaccutainers. The whole blood were used for estimation of Total Erythrocyte Count (TEC-millions/µL), Hemoglobin (Hb-g%) concentration and platelet count by using automatic whole blood analyzer (*CPC Diagnostics)* at Biological E. Ltd., Hyderabad and serum vaccutainers were allowed clot 3-4 hours and stored at refrigerating temperature for overnight, later centrifuged at 20k RPM for 10 minutes to separate the serum. The serum was collected into eppendorf tubes and stored at -20$^0$C, later thawed at room temperature and used for serum biochemical parameters by using semi-automatic biochemical analyzer (*CPC Diagnostics)* at Biological E. Ltd., Hyderabad by using *I-chem (thermo Scientific Inc.)* kits. The parameters were viz., Alanine amino transferase (ALT), Aspartate amino transferase (AST).

Data obtained (B.wt, Hematological and Biochemical) was subjected to statistical analysis by applying one way ANOVA and using statistical package for social sciences (SPSS) version 16.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P < 0.05$ (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

**Weekly body weight gain (g)**

The higher mean values in control group at 24, 48 and 72 hrs time intervals of experiment were recorded as 229.1 ± 2.5, 229.1 ± 2.5 and 229 ± 2.5 and significantly (P<0.05) lower mean values were recorded 186.167 ± 2.5, 179.167 ± 2.5 and169.000 ± 2.5 at 24, 48, and 72 hrs of time intervals among treated group animals. The reduction in body weight gain might be due to reduced feed and water intake as the animals started have showing sluggish movements after 24 hrs of PQ injection and at the end of experiment rats were under deep shallow abdominal breath and unable to move. Recorded results were depicted in the table.2 and fig.1. This observation is in accordance with the earlier studies of Igarshi *et al.,* (2000), Dinis-Olievera *et al.,* (2008) and Lalrautfela *et al.*(2014).

**Total Erythrocyte Count- TEC (millions/µL)**

Higher mean values (3.842 ± 0.03, 3.825 ± 0.03 and 3.812 ± 0.03) of TEC in control group at 24, 48 and 72 hrs of experiment was recorded. Significantly (P<0.05) lower mean values (3.447 ± 0.03, 3.410 ± 0.03 and 3.203 ± 0.03) was observed in treated group during 24, 48, 72 hrs of experiment. This difference could be due to the toxic action of PQ on haematopoiesis and extra medullary haemopoisis and may be due to the consequences of less feed intake which led to defficiency of essentail micro and macro nutrients which play major role in haematopoiesis. the obtained results were showed in the table.3 and fig.2.The variation found in the present experiment was in accordance with observations of several authors (Nagao *et al.,* 1994; Vuksa *et al.,* 1983; Akinloye *et al.,* 2011 and Lalrautfela *et al.,* 2014) who conducted the experiments in rats.

**Haemoglobin concentration- Hb (g%)**

Significantly (P<0.05) lower mean values of Hb was recorded as 12.078 ± 0.17, 11.792 ± 0.17 and 11.837 ± 0.17 at 24, 48 and 72 hrs comparively control group which was recorded as 12.867 ± 0.17, 12.867 ± 0.17 and 12.832 ± 0.17 at same time intervals of the experiment. Hypothatically these results are attributing to the consequeal cahnges in haematopoiesis led failure of haemoglobin (pigment) synthesis, this pigment is assocaited with micro-mineral like Fe and assocaited with biochemically the porphyrin ring foarmation. The PQ is usually damge the hepatic cells which led to impaired detoxification there by excreated throgh kidney and damge to tubules which influence the erythropoitin secretion. Hb results are showed in table.4 and fig.3.

**Platelet count**

Comparitively the platelet count showed higher mean values (1908.00 ± 32.2, 1920.83 ± 32.2 and 1903.67 ± 32.2) in control group than treated group which showed a significantly (P<0.05) lower mean values (1676.33 ± 32.2, 1171.05 ± 32.2 and 1043.05 ± 32.2) at 24, 48 and 72 hrs of time intervals. This change is also attributing to the haematopoises paticularly on bonemarrow. The results were depicted in the table.5 and fig.4.

**SERUM BIOCHEMISTRY**

**Aspartate Transaminase- AST (IU/L)**

The AST mean values in control group is 298.533 ± 7.40, 299.133 ± 7.40 and 299.133 ± 7.40 and significantly (P<0.05) higher mean values 399.450 ± 7.40, 404.667 ± 7.40 and 404.667 ± 7.40 was observed in treated group at 24, 48 and 72 hrs of time intervals. The AST values are attributing that PQ has damaged the liver parenchyma. The results are showed in table.6 and graphically in fig.5. These observations are in accordance with Attia and Nasr (2009), Akinloye *et al.,* (2011), Ahmad *et al.*, (2013) and Lalrautfela *et al.,* (2014). Authors have attributed that a significant rise in AST and ALT levels were suggestive of PQ induced hepato toxicity.

**Alanine Transaminase- ALT (IU/L)**

Significantly (P<0.05) higher mean values were recorded among treated group at different time intervals as 106.450 ± 3.13, 112.667 ± 3.13 and 110.600 ± 3.13 when caomapred with controle in which the values are showed as 69.567 ± 3.13, 69.667 ± 3.13 and 70.433 ± 3.13. The elevated levles of AST is also suggestive of PQ toxicity when compared with control. All the results were presented in table.7 and graphycally showed in fig.6. Dere and Polat (2001) were also reported increase in GOT and decrease in GPT after intraperitoneal administration of PQ (20 mg/kg b.wt) at 2, 4, 8, 16, 32 and 64 hours intervals and concluded that this could be due to hepatic damage at different doses of PQ at different time intervals. In the present experiment a steadily raised transaminases is indicative of moderate to severe damage of hepatocytes at 24 mg/kg dose of PQ.

**Serum creatinine (mg/dL)**

The lower mean values of serum creatinine in control group was recorded (0.703 ± 0.02, 0.713 ± 0.02 and 0.702 ± 0.02) and significantly higher mean values (0.870 ± 0.02, 0.913 ± 0.02 and 0.895 ± 0.02) were recorded at 24, 48 and 72 hrs of time intervals of experiment. The results were depicted in table.8 and graphycally presented in fig.7. The augmented levels of creatinine in experimental rats (G-2) could be due the rapid absorption and distribution of PQ, as a result of hepato toxicity the highest concentration was reached to kidney for elimination, due to its renal toxic nature it was accumulated within the kidneys and lead to early and severe damage. Similar findings and explanation was published by previous authors (Akinloye *et al.,* 2011; Ogamba *et al.,* 2011 and Lalrautfela *et al.,* 2014. The increased levels of serum creatinine may also be due to the over production of ROS which in accordance with Singh *et al.* (2011).

| Time intervals | Control | Treated |
|---|---|---|
| 24 hrs | 229.1 ± 2.5 [a] | 186.167 ± 2.5 [b] |
| 48hrs | 229.1 ± 2.5 [a] | 179.167 ± 2.5 [b] |
| 72 hrs | 229.1 ± 2.5 [a] | 169 .000± 2.5 [c] |
| P Value | NS | * |

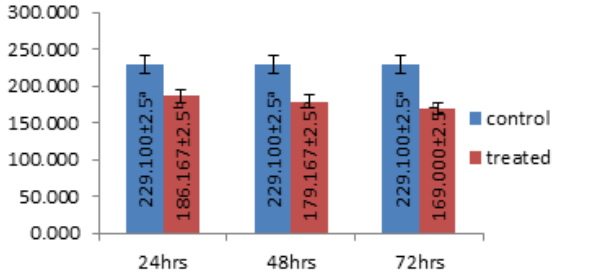


**Table 2 and Fig 1. Showing body weight gains (g) in different groups.**

| Time intervals | Control | Treated |
|---|---|---|
| 24 hrs | 3.842±0.03[a] | 3.447±0.03[b] |
| 48 hrs | 3.825±0.03[a] | 3.410±0.03[b] |
| 72 hrs | 3.812±0.03[a] | 3.203±0.03[c] |
| P Value | NS | * |

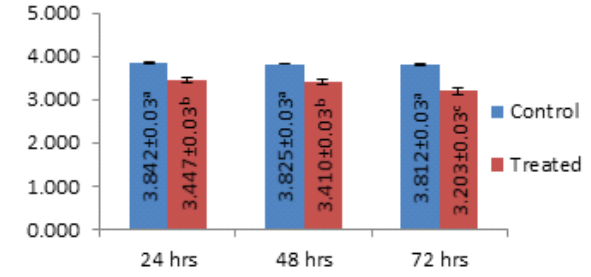


**Table 3. and Fig 2. Showing Total Erythrocyte Count (TEC - millions/µL) in different groups.**

| Time intervals | Control | Treated |
|---|---|---|
| 24 hrs | 12.867 ± 0.17[a] | 12.078 ± 0.17[b] |
| 48 hrs | 12.867 ± 0.17[a] | 11.792 ± 0.17[b] |
| 72 hrs | 12.832 ± 0.17[a] | 11.837 ± 0.17[b] |
| P Values | NS | * |

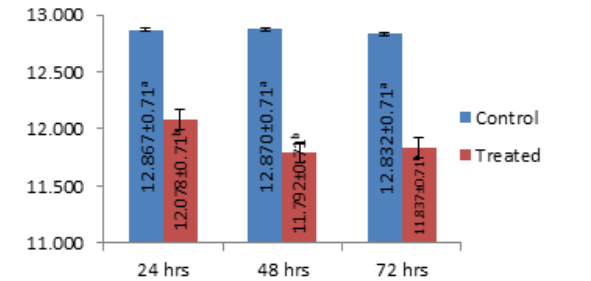


**Table 4 and Fig 3. Showing haemoglobin (Hb) concentration (g %) in different groups.**

| Time intervals | Control | Treated |
|---|---|---|
| 24 hrs | 1908.00±32.2[a] | 1676.33±32.2[b] |
| 48 hrs | 1920.83±32.2[a] | 1171.05±32.2[c] |
| 72 hrs | 1903.67±32.2[a] | 1043.05±32.2[d] |
| P Value | NS | * |

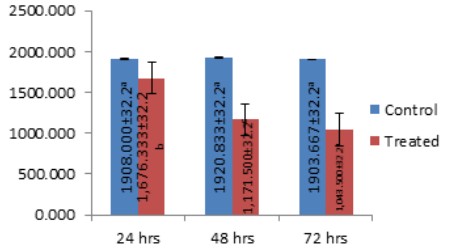


**Table 5 and Fig 4. Showing Total Platelets count in different groups.**

| Time Intervals | Control | Treated |
|---|---|---|
| 24 Hrs | 298.533±7.40[a] | 399.450±7.40[b] |
| 48 Hrs | 299.133±7.40[a] | 404.667±7.40[b] |
| 72 Hrs | 299.133±7.40[a] | 427.18±7.407.[c] |
| P Value | NS | * |

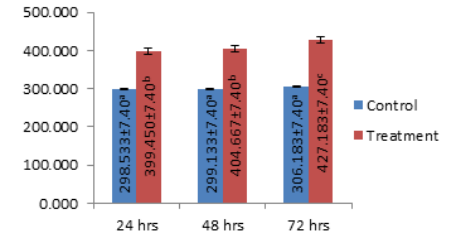


**Table 6 and Fig 5. Showing serum AST activity (IU/L) in different groups.**

| Time intervals | Control | Treated |
|---|---|---|
| 24 Hrs | 69.567±3.13[a] | 106.450±3.13[b] |
| 48 Hrs | 69.667±3.13[a] | 112.667±3.13[b] |
| 72 Hrs | 70.433±3.13[a] | 110.600±3.13[b] |
| P Values | NS | * |

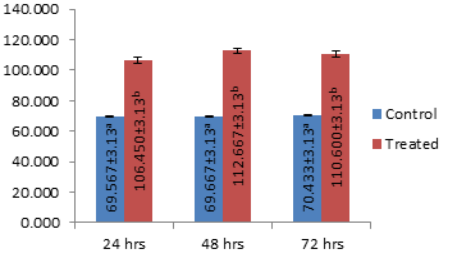


**Table 7 and Fig 6. Showing serum ALT activity (IU/L) in different groups.**

| Time intervals | Control | Treated |
|---|---|---|
| 24 Hrs | 0.703±0.02[a] | 0.870±0.02[b] |
| 48 Hrs | 0.713±0.02[a] | 0.913±0.02[b] |
| 72 Hrs | 0.702±0.02[a] | 0.895±0.02[b] |
| P Value | NS | * |

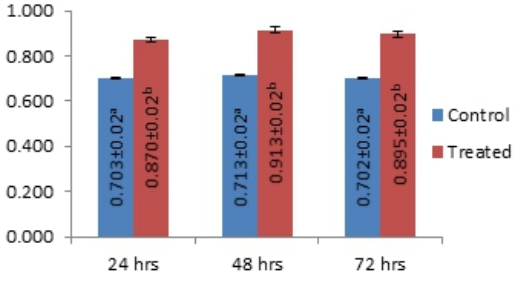


**Table 8. Showing and Fig 7. Showing serum Creatinine (mg/dL) in different groups.**

Values are Mean ± SE (n=6); one way ANOVA means with different superscripts in a column differ significantly at P<0.05 (*).

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