



Time-Dependent Evaluation of Diazinon-Induced Haematological Alterations and Their Amelioration by *Aloe barbadensis* in Albino Rats

Lata Fauzdar¹, Dr. Sonal Singh^{1&2}, Dr. Anand Pratap Singh^{*1}

¹Department of Zoology, Agra College, Agra, Affiliated to Dr. Bhimrao Ambedkar University, Agra, Uttar Pradesh, India

²Department of Zoology, D.S College, Aligarh, affiliated to Raja Mahendra Pratap Singh University, Aligarh, Uttar Pradesh, India

* Corresponding author. E-mail address: *anandmainpuri@gmail.com, latafoujdar9897@gmail.com

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ABSTRACT

The present experiment was designed to evaluate the time dependent hematological changes induced by repeated oral exposure of diazinon and protective role of *Aloe barbadensis* in male Wistar albino rats. The median lethal dose (LD₅₀) of diazinon was estimated to be approximately 300 mg/kg body weight, and one-tenth of this dose (30 mg/kg) was chosen for repeated exposure. The experimental animals were divided into control, diazinon, diazinon + *Aloe barbadensis* and recovery groups. Hematological parameters were assessed on 7th, 14th, 21st and 28th day. Hemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and erythrocyte sedimentation rate (ESR) were the parameters studied. Hematological indices showed progressive changes following exposure to Diazinon, characterized by decreased hemoglobin concentration, erythrocyte count, packed cell volume, MCH and MCHC and increased leucocyte count, MCV and ESR. The effects were further enhanced with increased duration of exposure indicating cumulative haematotoxic effects. Administration of *Aloe barbadensis* normalized several diazinon-induced abnormalities and the recovery group showed generally better improvement toward control values. Although these mechanisms were not directly investigated, the observed protective responses may be attributed to the antioxidant and cytoprotective constituents of *Aloe barbadensis*. The results indicated that repeated exposure of diazinon disturbs the hematological homeostasis and *Aloe barbadensis* might reduce the associated alterations. Further experimental validation is needed to identify the underlying protective mechanisms and to establish reproducibility in independent animal studies.

Introduction

Pesticides made synthetically are an important component of modern agriculture, and have contributed much to increasing crop production and controlling pests effectively. However, their indiscriminate and long-term use has raised serious concerns about environmental contamination and possible health hazards to non-target organisms. Among the different classes of pesticides organophosphorus compounds are well known for their insecticidal effectiveness and toxicological significance. Diazinon is an organophosphorus insecticide widely used in agriculture, horticulture and home for control of insect pests. The main toxic mechanism of its action consists in inhibition of acetylcholinesterase, which results in a massive accumulation of acetylcholine in the cholinergic synapses, leading to disorders in the functioning of the nervous system. Moreover, repeated exposure to diazinon can lead to systemic physiological changes including oxidative stress, cellular dysfunction and alterations in hematological and biochemical homeostasis (Costa, 2018; Mostafalou & Abdollahi, 2017).

Hematological parameters are sensitive indices of physiological health and toxicological responses in experimental animals. Changes in the concentration of hemoglobin, total erythrocyte count, total leucocyte count, packed cell volume and erythrocyte indices provide useful information regarding oxygen transport,

erythropoiesis, immune responses and cellular integrity. Exposure to organophosphorus pesticides may interfere with the normal hematopoietic processes. This may result in anemia, inflammatory responses and changes in blood cells characteristics. Such abnormalities may result from oxidative damage to erythrocyte membranes, disturbances in hemoglobin synthesis and changes in cellular survival. The systematic investigation of hematological indices is therefore an important way to understand pesticide-induced toxicity and to evaluate the progress of physiological disturbances during repeated exposure (Mostafalou & Abdollahi, 2017).

The growing scientific attention to bioactive compounds of plant origin has stimulated research on natural substances capable of reducing chemically-induced physiological disturbances. *Aloe barbadensis* Miller (*Aloe vera*) is a medicinal plant that contains a wide variety of phytochemical constituents and pharmacological properties. The leaf gel contains biologically active substances such as polysaccharides, flavonoids, phenolic compounds, vitamins, amino acids, enzymes and minerals. The antioxidant, anti-inflammatory and tissue-protective effects of these constituents were studied. Previous studies have indicated that under certain experimental conditions *Aloe vera* may aid in cellular protection through decreasing oxidative damage and aiding physiological homeostasis (Hamman, 2008; Reynolds & Dweck, 1999; Surjushe *et al.*, 2008).

The possible protective properties of *Aloe barbadensis* afford a scientific basis for evaluating its effects against diazinon-induced

hematological disturbances. However, protective responses may depend on pesticide concentration, duration of exposure, experimental conditions and treatment protocols. Thus, it is essential to study the temporal changes in blood parameters to understand the progression of toxicity and possible ameliorative responses.

The present study was aimed to evaluate the time-dependent hematological changes following repeated oral administration of diazinon in male Wistar albino rats and to study the possible protective effect of *Aloe barbadensis*. Hematological observations were made on days 7, 14, 21 and 28 and compared between control, diazinon-exposed, combined-treatment and recovery groups. Erythrocytic indices, leucocyte responses and erythrocyte sedimentation rate were given particular emphasis. Hence, the present comparative study is aimed to profile exposure duration-dependent alterations and to ascertain if *Aloe barbadensis* administration is associated with amelioration of diazinon-induced hematological dysfunctions. The study would contribute to the experimental elucidation of plant-derived protective interventions against pesticide-induced physiological disruptions.

Materials and Methods

Experimental Animals and Housing Conditions- The present experimental investigation was conducted using healthy adult male Wistar albino rats (*Rattus norvegicus*), approximately 8–10 weeks old, with an initial body weight ranging from 150 to 200 g. Animals were obtained from a CPCSEA-registered breeding facility and maintained under controlled laboratory conditions. All animals were acclimated for 14 days prior to experimentation to minimize environmental and physiological stress. The animal house was kept at $22 \pm 2^\circ\text{C}$, 55–65% relative humidity and a light–dark cycle of 12 hours. Standard laboratory pellet feed and drinking water were given ad libitum. All experimental procedures were performed in accordance with the institutional animal ethical guidelines and principles of laboratory animal welfare (National Research Council, 2011).

Experimental Toxicant and LD₅₀ Determination- The experimental toxicant chosen was diazinon, an organophosphate insecticide. The acute oral toxicity test was conducted with 5 dose concentrations of 100, 200, 300, 400 and 500 mg/kg body weight and 6 animals were selected for each dose group. Mortality and clinical manifestations occurred within 14 days after administration. The median lethal dose (LD₅₀) was determined by the log-dose probit method according to Finney (1971). Experimental observations indicated an approximate LD₅₀ of 300 mg/kg body weight. Therefore, one tenth of the estimated LD 50, that is 30 mg/kg body weight was chosen for repeated oral application for a maximum experimental period of 28 days.

Preparation of *Aloe barbadensis* Extract- Fresh matured healthy leaves of *Aloe barbadensis* Miller were obtained from pesticide free plants and were authenticated by qualified botanist. The leaves were washed thoroughly with tap water and then with distilled water to remove the contaminants. The outer margins and the green epidermal portions were removed and the transparent inner gel was carefully separated and homogenized with an electric blender. The resulting material was filtered through sterile muslin cloth and filter paper to obtain a crude aqueous extract. The prepared extract was stored in sterile amber-coloured containers at approximately 4°C until administration. This extraction approach was intended to preserve the naturally occurring phytochemical constituents of *Aloe gel* (Hamman, 2008; Reynolds & Dweck, 1999).

Experimental Design and Haematological Assessment- Experimental animals were assigned to four treatment categories: control, diazinon-exposed, diazinon plus *Aloe barbadensis*-treated, and *Aloe*-associated recovery groups. Hematological observations were made at 7, 14, 21 and 28 days. Blood samples were processed by standard laboratory procedures and evaluated for hemoglobin concentration, total erythrocyte count, total leucocyte count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and erythrocyte sedimentation rate. These indices were used to evaluate erythrocytic alterations and associated physiological responses (Jain, 1986).

Statistical Analysis- Experimental results were expressed as mean $\pm$ standard deviation (SD). Differences among experimental groups were assessed using one-way analysis of variance (ANOVA),

followed by Tukey's post hoc multiple-comparison test. Statistical significance was evaluated at $P < 0.05$ (Tukey, 1949).

Results and Discussion

The present observation tables record progressive changes across the four exposure periods. The complete original summary tables for eight haematological endpoints are reproduced below, preserving the numerical observations and reported significance annotations.

Table 1 Haemoglobin (Hb) at 7, 14, 21 and 28 day

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	15.68 $\pm$ 0.42 ^a	15.74 $\pm$ 0.39 ^a	15.81 $\pm$ 0.36 ^a	15.86 $\pm$ 0.41 ^a
Diazinon 30 mg/kg b.w.	13.92 $\pm$ 0.47 ^b	12.48 $\pm$ 0.44 ^c	11.42 $\pm$ 0.39 ^d	10.81 $\pm$ 0.43 ^e
Diazinon 30 mg/kg + <i>Aloe barbadensis</i>	14.68 $\pm$ 0.40 ^{ab}	14.22 $\pm$ 0.36 ^b	13.81 $\pm$ 0.41 ^c	13.42 $\pm$ 0.38 ^c
Diazinon 30 mg/kg + Recovery with <i>Aloe barbadensis</i>	14.94 $\pm$ 0.36 ^a	14.57 $\pm$ 0.34 ^b	14.13 $\pm$ 0.37 ^{bc}	13.86 $\pm$ 0.35 ^c
F-value	28.64	52.31	86.47	112.85
P-value	<0.0001	<0.0001	<0.0001	<0.0001

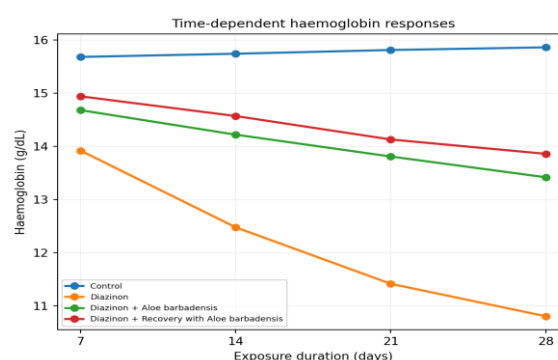


Figure 1 Time-dependent changes in haemoglobin (Hb) concentration in male Wistar albino rats following repeated oral exposure to diazinon and treatment with *Aloe barbadensis* at 7, 14, 21, and 28 days. The graph represents mean haemoglobin values for the control, diazinon-treated, diazinon plus *Aloe barbadensis*, and *Aloe*-associated recovery groups, based on the experimental observations.

At day 28, the reported control value was 15.86 ± 0.41^a , the diazinon-only value was 10.81 ± 0.43^e , the concurrent *Aloe* value was 13.42 ± 0.38^c , and the recovery-group value was 13.86 ± 0.35^c . These observations indicate a treatment-associated difference;

Table 2. Total erythrocyte count (TEC) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	8.42 $\pm$ 0.24 ^a	8.45 $\pm$ 0.22 ^a	8.48 $\pm$ 0.20 ^a	8.51 $\pm$ 0.23 ^a
Diazinon (30 mg/kg b.w.)	7.54 $\pm$ 0.21 ^b	6.92 $\pm$ 0.24 ^c	6.31 $\pm$ 0.19 ^d	5.86 $\pm$ 0.18 ^e
Diazinon + <i>Aloe barbadensis</i>	7.96 $\pm$ 0.20 ^b	7.61 $\pm$ 0.18 ^b	7.24 $\pm$ 0.22 ^c	6.98 $\pm$ 0.20 ^c
Diazinon + Recovery with <i>Aloe barbadensis</i>	8.18 $\pm$ 0.22 ^a	8.02 $\pm$ 0.20 ^{ab}	7.82 $\pm$ 0.21 ^b	7.63 $\pm$ 0.19 ^b
F-value	31.84	58.72	97.15	138.63
P-value	<0.0001	<0.0001	<0.0001	<0.0001

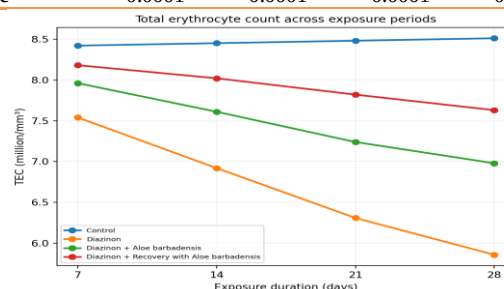


Figure 2 Time-dependent changes in total erythrocyte count (TEC) in

male Wistar albino rats following repeated oral exposure to diazinon and treatment with *Aloe barbadensis* at 7, 14, 21, and 28 days. The graph presents mean TEC values for the control, diazinon-treated, diazinon plus *Aloe barbadensis*, and Aloe-associated recovery groups, based on the corresponding experimental observation table. At day 28, the reported control value was 8.51 ± 0.23^a , the diazinon-only value was 5.86 ± 0.18^c , the concurrent Aloe value was 6.98 ± 0.20^c , and the recovery-group value was 7.63 ± 0.19^b . These observations indicate a treatment-associated difference; the reported analysis should be verified against raw records.

Table 3. Total leucocyte count (TLC) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	8.12 ± 0.28 ^a	8.18 ± 0.26 ^a	8.23 ± 0.24 ^a	8.26 ± 0.27 ^a
Diazinon (30 mg/kg b.w.)	9.46 ± 0.31 ^b	10.82 ± 0.34 ^c	12.14 ± 0.37 ^d	13.58 ± 0.41 ^e
Diazinon + Aloe barbadensis	8.96 ± 0.27 ^b	9.42 ± 0.29 ^b	9.86 ± 0.31 ^c	10.34 ± 0.33 ^c
Diazinon + Aloe barbadensis Recovery	8.58 ± 0.25 ^a	8.74 ± 0.24 ^{ab}	8.96 ± 0.27 ^b	9.18 ± 0.28 ^b
F-value	29.74	56.88	91.35	145.82
P-value	<0.0001	<0.0001	<0.0001	<0.0001

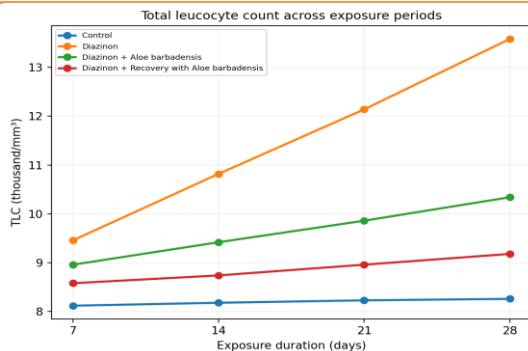


Figure 3 Time-dependent changes in total leucocyte count (TLC) in male Wistar albino rats following repeated oral exposure to diazinon and treatment with *Aloe barbadensis* at 7, 14, 21, and 28 days. The graph presents mean TLC values for the control, diazinon-treated, diazinon plus *Aloe barbadensis*, and Aloe-associated recovery groups, based on the corresponding experimental observation table. At day 28, the reported control value was 8.26 ± 0.27^a , the diazinon-only value was 13.58 ± 0.41^e , the concurrent Aloe value was 10.34 ± 0.33^c , and the recovery-group value was 9.18 ± 0.28^b .

Table 4. Packed cell volume (PCV) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	44.82 ± 1.21 ^a	45.14 ± 1.18 ^a	45.46 ± 1.16 ^a	45.82 ± 1.24 ^a
Diazinon (30 mg/kg b.w.)	39.76 ± 1.34 ^b	36.82 ± 1.28 ^c	33.48 ± 1.22 ^d	30.26 ± 1.18 ^e
Diazinon + Aloe barbadensis (Treatment)	41.62 ± 1.26 ^b	40.14 ± 1.22 ^b	38.84 ± 1.18 ^c	37.56 ± 1.14 ^c
Diazinon + Aloe barbadensis (Recovery)	43.16 ± 1.18 ^{ab}	42.58 ± 1.16 ^b	41.84 ± 1.14 ^b	41.22 ± 1.12 ^b
F-value	41.62	68.84	112.73	169.54
P-value	<0.0001	<0.0001	<0.0001	<0.0001

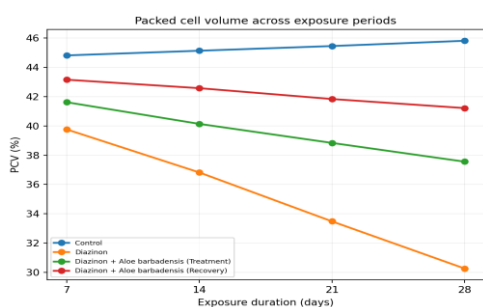


Figure 4 Time-dependent changes in packed cell volume (PCV) in male Wistar albino rats following diazinon exposure and *Aloe barbadensis* treatment at 7, 14, 21, and 28 days. Values represent group means obtained from the corresponding experimental observation table. At day 28, the reported control value was 45.82 ± 1.24^a , the diazinon-only value was 30.26 ± 1.18^e , the concurrent Aloe value was 37.56 ± 1.14^c , and the recovery-group value was 41.22 ± 1.12^b .

Table 5. Mean corpuscular volume (MCV) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	54.62 ± 1.42 ^a	54.84 ± 1.38 ^a	55.06 ± 1.34 ^a	55.24 ± 1.29 ^a
Diazinon (30 mg/kg b.w.)	58.84 ± 1.56 ^b	61.72 ± 1.63 ^c	64.86 ± 1.71 ^d	68.12 ± 1.82 ^e
Diazinon + Aloe barbadensis (Treatment)	56.94 ± 1.48 ^b	58.42 ± 1.46 ^b	59.76 ± 1.52 ^c	61.18 ± 1.57 ^c
Diazinon + Aloe barbadensis (Recovery)	55.82 ± 1.44 ^a	56.38 ± 1.41 ^{ab}	57.16 ± 1.46 ^b	58.02 ± 1.49 ^b
F-value	38.52	72.36	119.84	181.42
P-value	<0.0001	<0.0001	<0.0001	<0.0001

At day 28, the reported control value was 55.24 ± 1.29^a , the diazinon-only value was 68.12 ± 1.82^e , the concurrent Aloe value was 61.18 ± 1.57^c , and the recovery-group value was 58.02 ± 1.49^b .

Table 6. Mean corpuscular haemoglobin (MCH) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	18.62 ± 0.64 ^a	18.78 ± 0.61 ^a	18.92 ± 0.58 ^a	19.08 ± 0.56 ^a
Diazinon (30 mg/kg b.w.)	16.84 ± 0.58 ^b	15.68 ± 0.54 ^c	14.42 ± 0.49 ^d	13.24 ± 0.46 ^e
Diazinon + Aloe barbadensis (Treatment)	17.54 ± 0.56 ^b	16.98 ± 0.52 ^b	16.42 ± 0.50 ^c	15.86 ± 0.48 ^c
Diazinon + Aloe barbadensis (Recovery)	18.16 ± 0.60 ^a	17.82 ± 0.56 ^{ab}	17.46 ± 0.53 ^b	17.12 ± 0.50 ^b
F-value	35.84	69.42	118.26	176.83
P-value	<0.0001	<0.0001	<0.0001	<0.0001

At day 28, the reported control value was 19.08 ± 0.56^a , the diazinon-only value was 13.24 ± 0.46^e , the concurrent Aloe value was 15.86 ± 0.48^c , and the recovery-group value was 17.12 ± 0.50^b .

Table 7. Mean corpuscular haemoglobin concentration (MCHC) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	33.84 ± 0.82 ^a	34.02 ± 0.78 ^a	34.18 ± 0.74 ^a	34.36 ± 0.71 ^a
Diazinon (30 mg/kg b.w.)	31.26 ± 0.76 ^b	29.84 ± 0.72 ^c	28.42 ± 0.68 ^d	27.08 ± 0.64 ^e
Diazinon + Aloe barbadensis (Treatment)	32.42 ± 0.78 ^b	31.84 ± 0.74 ^b	31.12 ± 0.70 ^c	30.48 ± 0.66 ^c
Diazinon + Aloe barbadensis (Recovery)	33.18 ± 0.80 ^a	32.86 ± 0.76 ^{ab}	32.48 ± 0.72 ^b	32.12 ± 0.69 ^b
F-value	37.48	71.62	116.84	178.26
P-value	<0.0001	<0.0001	<0.0001	<0.0001

At day 28, the reported control value was 34.36 ± 0.71^a , the diazinon-only value was 27.08 ± 0.64^e , the concurrent Aloe value was 30.48 ± 0.66^c , and the recovery-group value was 32.12 ± 0.69^b .

Table 8. Erythrocyte sedimentation rate (ESR) at 7, 14, 21 and 28 days

Experimental Groups	7 Days	14 Days	21 Days	28 Days
Control	2.14 ± 0.18 ^a	2.18 ± 0.16 ^a	2.22 ± 0.15 ^a	2.26 ± 0.17 ^a
Diazinon (30 mg/kg b.w.)	3.42 ± 0.22 ^b	4.26 ± 0.28 ^c	5.18 ± 0.31 ^d	6.12 ± 0.34 ^e

Diazinon + Aloe barbadensis (Treatment)	2.96 0.20 ^b	±	3.42 0.24 ^b	±	3.86 0.27 ^c	±	4.32 0.29 ^c	±
Diazinon + Aloe barbadensis (Recovery)	2.48 0.19 ^a	±	2.72 0.20 ^{ab}	±	2.96 0.22 ^b	±	3.18 0.24 ^b	±
F-value	30.84		57.96		98.72		152.43	
P-value	<0.0001		<0.0001		<0.0001		<0.0001	

At day 28, the reported control value was 2.26 ± 0.17^a , the diazinon-only value was 6.12 ± 0.34^c , the concurrent Aloe value was 4.32 ± 0.29^c , and the recovery-group value was 3.18 ± 0.24^b .

The present investigation demonstrates that repeated oral exposure to diazinon produces progressive alterations in selected haematological parameters of male Wistar albino rats, while administration of *Aloe barbadensis* is associated with attenuation of several abnormalities. The exposure duration dependent changes recorded at 7, 14, 21 and 28 days suggest that prolonged administration of pesticides may lead to progressive disruption of the hematological homeostasis. These results are in accordance with the known toxicological properties of organophosphorus pesticides, which primarily act by inhibiting acetylcholinesterase but may also cause oxidative stress, inflammatory reactions, and cell dysfunction (Costa, 2018; Mostafalou & Abdollahi, 2017).

The concentration of hemoglobin is an important indicator of the oxygen-carrying capacity of the blood and the functional state of erythrocytes. In the present study, repeated diazinon exposure was associated with progressive decrease in hemoglobin concentration with more significant reductions following long-term treatment. The changes may be secondary to impaired erythrocyte function, reduced hemoglobin synthesis, or increased destruction of circulating red blood cells. Previous toxicological studies have shown that organophosphorus pesticides may cause the generation of reactive oxygen species that result in oxidative damage of cell membranes and disturbance of the normal physiological functions (Abdollahi *et al.*, 2004; Mostafalou & Abdollahi, 2017). The present findings demonstrate changes in hemoglobin concentration but do not directly establish the mechanisms underlying these changes.

Total erythrocyte count was also found to be decreased following administration of diazinon. Erythrocytes are particularly prone to oxidative damage because of the presence of polyunsaturated fatty acids in the membranes and their constant exposure to oxygen during circulation. Excessive oxidative stress can alter membrane permeability and thus diminish erythrocyte stability and hasten cell death. Lipid peroxidation is described as an important mechanism by which reactive oxygen species can damage the integrity of biological membranes (Halliwell and Gutteridge, 2015). Therefore, the reduced erythrocyte counts found in the present study may be consistent with oxidative membrane damage or erythropoiesis disturbances. However, the proposed mechanisms would require direct measurements of the erythrocyte fragility, bone marrow activity and oxidative biomarkers.

Packed cell volume also decreased progressively in animals exposed to diazinon supporting the observed reductions in hemoglobin concentration and erythrocyte count. Packed cell volume indicates the percentage of erythrocytes in circulating blood and is most often used in the evaluation of anemic states and physiological disturbances. A concomitant decrease in hemoglobin, erythrocyte count and packed cell volume may suggest a breakdown of erythrocytic homeostasis. Similar hematological abnormalities have been reported in experimental toxicology where chemical exposure may affect the survival of red blood cells and blood-forming tissues (Jain, 1986; Klaassen, 2019). The combined analysis of these parameters thus provides a broader perspective of the haematotoxic effects of repeated administration of diazinon.

Changes in erythrocyte indices further indicate on hematological disturbances. In the present study, alterations in mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were observed after diazinon exposure. These indices give an idea of the size of the erythrocytes and the amount of hemoglobin. A high mean corpuscular volume and low mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration may indicate disturbances in erythrocyte characteristics and hemoglobin distribution. However, classification of the resulting anemia calls for careful interpretation of the full hematological profile including morphological examination of

peripheral blood smears. Therefore the observed changes must be taken as a pointer toward an altered erythrocyte physiology and not as a conclusive evidence of any particular anemia subtype (Jain, 1986). Repeated diazinon exposure resulted in increased total leucocyte count compared to erythrocytic parameters. Leukocytes are important constituents of the immune defense and are involved in inflammatory and stress related responses. Increased white blood cell counts may be considered as physiological reactions to tissue damage, systemic stress or inflammatory stimuli. Organophosphorus pesticides exposure has been implicated in inflammatory pathways and oxidative disturbances that may affect immune regulation (Mostafalou & Abdollahi, 2017). But high leucocyte counts do not necessarily indicate that there is a specific inflammatory mechanism. Further studies with differential leucocyte counts, inflammatory cytokines and tissue level analysis would provide more clarity about the immunological effects of diazinon exposure.

During the experimental period, an increase in erythrocyte sedimentation rate was recorded, which may indicate changes in the systemic inflammatory or physiological conditions. ESR is a non-specific hematologic marker that is affected by plasma proteins, red blood cell characteristics, and inflammation. The increased ESR values may therefore be associated with tissue injury or change in blood composition. The concurrent increase in leucocyte count and ESR in the present study suggests that there is a probable inflammatory or stress-related response following repeated pesticide administration. However, these observations do not independently confirm inflammation and further evaluation of inflammatory mediators would be needed to establish the biological pathways responsible. Important findings of present investigation was the relatively improved hematological profile observed in animals treated with *Aloe barbadensis*. Overall, the Aloe-associated treatment groups had higher hemoglobin concentration, erythrocyte count and packed cell volume than animals treated with diazinon alone. Leucocyte counts, erythrocyte indices and ESR values also showed a trend toward control values. These results suggest that administration of *Aloe barbadensis* may ameliorate some of the blood disturbances associated with repeated exposure to diazinon.

The protective action of *Aloe barbadensis* may be due to its phytochemical composition. Antioxidant and tissue-protective properties of polysaccharides, phenolic compounds, flavonoids, vitamins and other biologically active constituents have been studied (Hamman, 2008; Reynolds & Dweck, 1999). Aloe gel contains polysaccharides. Surjushe *et al.* (2008) also reviewed the possible antioxidant and anti-inflammatory activities of Aloe vera along with its various biological properties. Such characteristics provide plausible explanation for the observed improvement in hematological indices. However, the present study did not directly measure reactive oxygen species, lipid peroxidation, antioxidant enzyme activities or inflammatory cytokines. Consequently, the protective mechanisms remain hypothetical and require further experimental confirmation. The comparatively favourable responses observed in the recovery group suggest that some diazinon-associated haematological abnormalities may be reversible under the experimental conditions. However, the interpretation of the recovery requires an accurate record of the treatment chronology, extract concentration, the duration of administration, and the time between pesticide withdrawal and sample collection. It is also important to consider the possibility of spontaneous physiological recovery when evaluating treatment-associated improvements. In general, the present results indicate that multiple exposure to diazinon is associated with gradually changed erythrocytic and leucocytic indices in male albino rats. Administration of *Aloe barbadensis* was associated with partial normalization of some parameters, suggesting a possible haematoprotective activity. But these results must be interpreted according to the experimental design and the measurements that are available. Further studies with validated phytochemical characterization, oxidative stress biomarkers, histopathological examination and controlled treatment protocols are needed to elucidate mechanisms and reproducibility of the observed protective responses.

Conclusion

The present experimental investigation demonstrates that repeated oral administration of diazinon is associated with progressive haematological alterations in male Wistar albino rats. The changes observed over 7, 14, 21, and 28 days indicate an exposure-duration-

dependent disturbance in normal blood physiology. The decline in hemoglobin concentration, total erythrocyte count and packed cell volume in Diazinon treated animals suggested impairment of erythrocytic homeostasis and probably diminished oxygen-carrying capacity. Other indices of disturbances in erythrocyte characteristics are changes in mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Meanwhile, increased total leucocyte count and erythrocyte sedimentation rate may point toward possible inflammatory or stress-related physiological responses after repeated pesticide exposure. The observed improvement in the hematological profiles in *Aloe barbadensis* supplemented animals suggests its capacity to ameliorate selected diazinon associated abnormalities. The increase of the erythrocytic indices and the tendency of the leucocyte counts and the erythrocyte sedimentation rate toward the control values may suggest a possible protectory effect of Aloe administration. These observations are in agreement with reported antioxidant and cytoprotective properties of *Aloe barbadensis*. However, the underlying mechanisms cannot be established conclusively due to lack of direct evaluation of oxidative stress biomarkers, inflammatory mediators and erythrocyte membrane integrity. The results emphasize the importance of exposure duration in the determination of severity of pesticide-associated hematological disturbances and highlight the possible relevance of plant-derived substances in experimental toxicology. However, the interpretation requires rigorous verification of numerical observations, treatment chronology, dosage of Aloe extract and statistical analyzes. Further investigations including the preparation of standardized extracts, use of appropriate experimental controls, assessments of oxidative stress and histopathological examinations are recommended. The present study demonstrates that repeated exposure to diazinon produces adverse effects on the hematological homeostasis, while administration of *Aloe barbadensis* may contribute to partial improvement of some selected blood parameters. The findings provide a preliminary experimental basis for further investigation of the protective responses associated with Aloe against organophosphorus pesticide toxicity.

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