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Research Article

Acute Toxicological Assessment and Haematological Effect of *Plumbago zeylanica*, Linn in Rodents

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ABSTRACT
Objectives: To evaluate the acute toxicity and changes in haematological indices induced by ethylacetate fraction of methanol extract of Plumbago zeylanica root.
Methods: Acute toxicity was assessed by administering orally single low doses of extract (2000-5000 mg/kg) and single high doses (10000-20000 mg/kg) to different groups of 11 mice; and by administering intraperitoneally single high doses (500-1000 mg/kg) and single low doses (100-400 mg/kg) to another groups of 20 mice. These were observed for 48 h. The toxic effect on the marrow was assessed by administering orally 100, 200, 400 mg/kg body weight of extract to 32 rats for 28 day.
Results: No mortality recorded in mice at dose 20,000 mg/kg body weight by the oral route, while 50% mortality was recorded in 100 mg/kg body weight by the intraperitoneal route. The haematological indices value was highest in white blood cells (7.64±0.54 x 109/L - 80% at 400 mg/kg b.wt.), followed by platelets (654.38±53.17 x 109/L - 49% at 100 mg/kg b.wt.). Slight increases ($p<0.05$) observed in mean corpuscular volume (55.96±1.10 fl - 7% at 100 mg/kg b.wt.), mean corpuscular haemoglobin (20.68±0.38 Pg - 8% at 100 mg/kg b.wt.) and mean corpuscular haemoglobin concentration (381.62±3.01 g/L - 2% at 200 mg/kg b.wt.), while there were reduction in red blood cell count (5.94±0.33 x 1012/L - 26% at 200 mg/kg b.wt.), haemoglobin (118.75±6.96 g/L - 18.5% at 200 mg/kg b.wt.) and packed cell volume (31.71±1.67 % - 19% at 200 mg/kg b.wt.).
Conclusion: This study suggests that EAME has a degree of toxic effect.

INTRODUCTION

On a global scale, medicinal plants are mainly used as crude drugs and extracts. Phytochemicals from plants have been used by humans to treat infections, health disorders and illness.[1] World Health Organization (2003) highlighted the importance of quality control, safety and proper validation of medicinal plants to prevent wrong use of plants species.[2] Other issues raised were contamination by chemicals or unwanted foreign substances, adulteration, factors that may induce over dose, wrong use by health providers and consumers, and undesirable interaction by various medicinal plants.[2]

Plumbago belongs to the plants family of plumbaginaceae which is native to warm temperate and tropical regions of the world. Common names include plumbago and leadwort. *Plumbago zeylanica* is distributed throughout most of the tropics and subtropics, growing in deciduous woodland, savannas and shrub lands from sea level up to 2000 metres altitude.[3] In West Africa, the root or the leaves crushed with lemon juice, are used as a counter-irritant and vesicant. In Nigeria, the roots pounded with vegetable oil are used as a treatment for rheumatic swellings.[3] Other parts of Africa use the paste of the root in vinegar, milk and water to treat influenza and black water fever. The root decoction is used in the treatment of shortness of breath, inflammation in the mouth, throat and chest, including diarrhea and dyspepsia.[4,5] In India, *P. zeylanica* commands an important place among medicinal herbs since ancient times. In Charaka Samhita (an important work on Ayurvedic system of medicine in India) the plant has been categorized as an appetizer, antidyspepsia, antihaemorrhoizi, and analgesic,[6]

Research has been conducted on several solvents extracts of the plant and has been reported to possess pharmacological activities. These properties included alcohol or ethanol extracts that show anti-inflammatory, antibacterial, hyperglycaemic activities.[7-10] Hexane extracts of *P. zeylanica* were reported to show activity against canine distemper virus and as antioxidant.[11,12] Methanol extracts also have activity against skin diseases and hepatoprotective activity.[13,14] The petroleum ether extracts were reported to have biological activities such as antifertility [15] and anti-

inflammatory.[15,16] Other solvents extracts of *P. zeylanica* reportedly possess biological activities, for example ethylacetate extract for antiarthritic and antioxidant.[17,18]

The increase in the use of herbal medicines therefore necessitates a thorough scientific evaluation of toxicological properties of these herbs. In case of *Plumbago zeylanica*, reports on its toxicological properties are scanty especially, the ethylacetate fraction from methanolic extract of the plant. Therefore, it is important to have a thorough assessment of its toxicity as well as the toxic effects on various haematological parameters.

MATERIALS AND METHODS

Collection and identification of plant materials

The roots of *Plumbago zeylanica* were collected at Babajakan village, Ayedande Local Government Area, Osun State, Nigeria in the months of June-August, 2010 and 2011. The plant identification and authentication has been done as reported by Olagunju *et al*.[19] and Mr. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The voucher specimen has been deposited at the IFE Herbarium with code QC 488.

Preparation of ethylacetate extract of *P. zeylanica*

Roots were removed from the stalks and gently but thoroughly washed with tap water to remove sands. Cleansed roots were spread on black polythene on the laboratory table for 7 days to air-dry. It was further oven-dried completely at 35°C overnight. This was then ground to powder with a local grinder. The powdered *P. zeylanica* root (700 g) was soaked in 2.6 L 70% (v/v) methanol for 48 hours and then filtered with a piece of white nylon cloth. The residue was re-extracted five more times with the same solvent and time duration till the extract became colourless. The filtrates were combined and allowed to settle and decanted to obtain a clear solution of extract. The filtrates were pooled together and concentrated on a rotary vacuum evaporator. About 10 mL of extract was taken for phytochemical screening while the remaining extract was evaporated to complete dryness at 30°C rotary evaporator to obtain the methanol extract of *P. zeylanica* (ME), a dark-brown residue. About 30 g of ME was dissolved in 300 mL water : methanol (4:1) and poured into 1.0 L separating funnel. This was partitioned with 150 mL ethylacetate, shaken vigorously to allow the solvent systems to separate into two layers as ethylacetate is immiscible with water. Compounds soluble in the upper ethylacetate layer (ethylacetate being lighter than water) were collected and the lower aqueous layer was extracted three more times. All fractions of ethylacetate were pooled together and known as the ethylacetate fraction from methanol extract of *P. zeylanica* (EAME). EAME was evaporated to complete dryness to obtain solid extracts which was stored in an air-tight container and placed in the bio-freezer.

Determination of lethal dose-LD_{50}

Acute toxicity was carried out according to the procedure described by Lorke and as modified by Ezeonwumelu *et al*. via two different routes of administration (oral and intraperitoneal).[20,21]

Animals

Forty (40) healthy male and female albino mice weighing between 20 and 26 g were purchased from the Animal House Unit, College of Medicine, University of Lagos, Idi-Araba, Surulere, Lagos, Nigeria. These mice were housed in standard aluminum cages with saw-dust on its floor to keep the animals warm, under clean environmental conditions (24 ± 1°C, with 75 ± 5% humidity and 12 h / 12 h light / dark cycles) and allowed to acclimatize for 14 days. They were fed with pelletized growers feed from Grand Cereals Nigeria Limited, a subsidiary of UAC Vital Feed, Km 17, Zawan Round about, Jos, Plateau State, Nigeria and tap water *ad libitum*.

Evaluation of LD_{50} (Oral administration)

In the first phase of the experiment, nine (9) males and females mice were randomly divided into three groups of three mice per group. Group I served as the control was administered orally with the vehicle 2% (v/v) Tween-20 while Group II and Group III were administered 2000 mg/kg body weight and 5000 mg/kg body weight ethylacetate extract (EAME) of *Plumbago zeylanica* respectively. These animals were then observed for half hour, 1 hour, 2 hours, 3 hours and, for 24 and 48 hours for any general behavioral pattern and mortality.

The second phase was undertaken based on the results of the first phase. In this experiment, two (2) animals were grouped into two groups of one (1) animal per group. Group I and Group II were administered 10,000 mg/kg body weight and 20,000 mg/kg body weight EAME respectively. These animals were observed at 0.5 h, 1 h, 2 h, 3 hours and, for 24 and 48 hours for any general behavioral pattern and mortality. The number of survivor was noted at the end of 24 and 48 hours to determine the LD50.The numerical mean of the smallest dose that killed a mouse and the highest dose that did not kill a mouse was taken as the mean lethal dose (LD50) of the extract.

Evaluation of LD_{50} (Intraperitoneal administration (*i.p*))

In the first phase of the experiment, twelve (12) male and female mice (20-26g) were randomly grouped into four, containing three (3) animals per group. Group I was administered intraperitoneally the vehicle 2% (v/v) Tween-20. Groups II, III and IV were administered intraperitoneally 500, 750 and 1000 mg/kg body weight EAF *P. zeylanica* respectively. The animals were observed at 0.5 h, 1 h, 2 h, 3 hand, for 24 and 48 h for any general behavioral pattern and mortality.

The second phase was undertaken based on the results obtained in the first phase. In this experiment, eight (8) mice were divided randomly into four groups containing two animals per group. Groups I, II, III and IV were administered intraperitoneally 100, 200, 300 and 400 mg/kg body weight EAME respectively. The animals were observed at 0.5 h, 1 h, 2 h, 3 h and, for 24 and 48 h for any general behavioral pattern and mortality. The number of survivor was noted at the end of 24 and 48 hours to determine the LD_{50}. The numerical mean of the smallest dose that killed a mouse and the highest dose that did not kill a mouse was taken as the mean lethal dose (LD_{50}) of the extract.

Evaluation of toxicity of ethylacetate fraction of *P. zeylanica* root on haematological indices

Animals

A total of thirty two (32) healthy male and female albino rats (Wistar strain) weighing between 130 g and 180 g were used for this study; they were purchased from the Animal House Unit, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. The rats were housed in Aluminum cages with saw-dust on its floor to absorb urine and faeces of animals, under clean environmental conditions (23 ± 1°C, with 80 ± 5% humidity and 12 h / 12 h light / dark cycles) and allowed to acclimatize for 14 days. They were fed with pelletized feed from Grand Cereals Nigeria Limited, a subsidiary of UAC Vital Feed, Km 17, Zawan Round about, Jos, Plateau State, Nigeria and given tap water *ad libitum*.

Grouping and treatment of experimental animals

The thirty two (32) rats were randomly distributed into four groups of eight rats per group. Group I served as Control and was administered orally using cannula with 1 mL 2% (v/v) Tween-20 (the vehicle). Groups II, III and IV were administered orally with 100, 200 and 400 mg/kg body weight EAME extract of P. zeylanica respectively. The extract was administered daily for 28 days. The animals were weighed before the commencement of administration and every 7 day of the experiment till the completion of extract administration. The amount of feed consumed was weighed daily while the level of water consumed was also measured daily till the end of administration. At the end of extract administration, the animals were fasted overnight.

Animal sacrifice and collection of samples

On the 29th day, rats were anaesthesized with chloroform. The rats were opened and blood samples were collected by cardiac puncture into a set of sample bottles containing ethylenediaminetetraacetic acid (EDTA) for determination of haematological parameters.

Evaluation of haematological parameters

The blood samples already collected into sample bottles containing ethelenediaminetetraacetic acid (EDTA) were used for haematological analysis using an autoanalyzer – MINDRAY Auto Haematology Analyzer, Model: BC – 3000 Plus, manufactured by Shenzhen Mindray Bio-Medical Electronics Co. Ltd, Mindray Building, Keji 12th Road South, Hi-tech Industrial Park, Nanshan, Shezhen, 518057 PR-China. The following haematological parameters; white blood cell (WBC), haemoglobin (HGB), red blood cell (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT) were determined and results expressed following the standard convention.

RESULTS

Acute toxicity and behavioural response of rats to EAME

The results of the effects of oral and intraperitoneal administrations in acute toxicity study of ethylacetate extract of methanolic extract of *Plumbago zeylanica* root in mice and changes in behavioural pattern after intraperitoneal administration during the acute toxicity study were presented in Tables 1-3. No mouse died within 48 hours of study after oral administration of ethylacetate extract and there was no clinical symptoms observed during the same period even with a very high dose of 20 g/kg body weight (Table 1). Therefore, the oral LD_{50} (the mean lethal dose of ethylacetate extract) was estimated to be higher than 20,000 mg/kg body weight. 100% death was recorded in mice within 24 hours after intraperitoneal administration of ethylacetate extract with a dose range of 300-1000 mg/kg body weight. However, a dose of 200 mg/kg body weight resulted in 50% death in mice within 24 hours and 100% within 48 hours while a dose of 100 mg/kg resulted in 50% death in mice but no death in 48 hours (Table 2). The intraperitoneal LD_{50} was estimated to be less than 100 mg/kg body weight. The clinical symptoms started manifesting in the mice within few minutes of intraperitoneal administration at a dose of 300 mg/kg body weight. The process of death started with restlessness followed by hyperactivity, then inactivity (paralysis) and finally, death (Table 3).

Effect of ethylacetate extract of methanol extract on haematological parameters

The effects of ethylacetate extract of *P. zeylanica* on haematological parameters in treated rats are shown in Figures 1-8. There was a non-significant increase ($p>0.05$) in white blood cell count with 100 and 200 mg/kgbwt until at the highest dose of 400 mg/kgbwt where 80% increase was observed compared with the control (Figure 1) whereas a significant decrease ($p<0.05$) was noticed in haemoglobin concentration and red blood cells count which was more pronounced with 400 mg/kgbwt (18.5%) and 200 mg/kgbwt (26%) of extract respectively (Figures 2 and 3). A decrease was observed in packed cell volume which was significant at dose 200 mg/kgbwt by 18.8% in treated rats (Figure 4). Slight increase was noticed in mean corpuscular volume and mean corpuscular haemoglobin showing highest effect with dose 100 mg/kgbwt by 7.2% and 7.7% respectively (Figures 5 and 6). The mean corpuscular haemoglobin concentration was increased marginally by 2.2% (200 mg/kgbwt) and 1.2% (400 mg/kgbwt) (Figure 7) while platelets were significantly increased in treated rats by 48.8% (100 mg/kgbwt) and 12% (200 mg/kgbwt) compared with the control (Figure 8).

Table 1: Effect of acute toxicity of oral administration of ethylacetate extract of methanol extract of *P. zeylanica* root in mice

No. of mice	Dose mg/kg/b. wt	No. of dead rats in 24 h	% of dead rats in 24 h	No. of dead rats in 48 h	% of dead rats in 48 h
PHASE 1					
3	0	0	0	0	0
3	2,000	0	0	0	0
3	5,000	0	0	0	0
PHASE 2					
1	10,000	0	0	0	0
1	20,000	0	0	0	0

Table 2: Effect of acute toxicity of intraperitoneal administration of ethylacetate extract of methanol extract of *P. zeylanica* root in mice

No. of mice	Dose mg/kg b. wt	No. of dead in 24 h	% of dead in 24 h	No. of dead in 48 h	% of dead in 48 h
PHASE 1					
3	0	0	0	0	0
3	500	3	100	-	-
3	750	3	100	-	-
3	1000	3	100	-	-
PHASE 2					
2	100	1	50	-	
2	200	1	50	1	100
2	300	2	100	-	-
2	400	2	100	-	-

Table 3: Changes in behavioural pattern after interperitonial administration of ethylacetate extract of methanol extract of *P. zeylanica* in mice

Duration	Dose 0 mg/kgbwt	Dose 100 mg/kgbwt	Dose 200 mg/kgbwt	Dose 300 mg/kgbwt	Dose 400 mg/kgbwt	Dose 500 mg/kgbwt	Dose 750 mg/kgbwt	Dose 1000 mg/kgbwt
5 min	Normal	Normal	Normal	Restlessness	Restlessness	Restlessness	Hyper-active	Aggressive
10min	Normal	Normal	Normal	Restlessness	Restlessness	Hyperactive	Inactive & quick breathing	Convusions & death
30min	Normal	Normal	Restlessness	Restlessness	Hyperactive	Inactive	1 death	
1 hr	Normal	Normal	Inactive	Inactive	Inactive & Hyperventillation followed by apnoea	1 death	2 death	
2 hr	Normal	Normal	Inactive	Inactive	1 death	1 death		
3 hr	Normal	Chocking sound	Inactive	1 death				
6 hr	Normal	Inactive	Inactive		1 death	1 death		
24 hr	Normal	1 death	1 death	1 death				
48 hr	Normal		1 death					

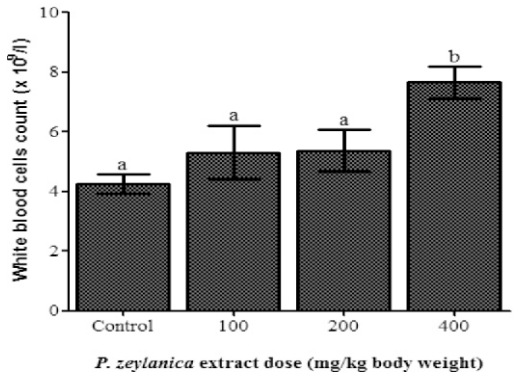

Figure 1: Effect of ethylacetate extract of *P. zeylanica* on white blood cells count. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

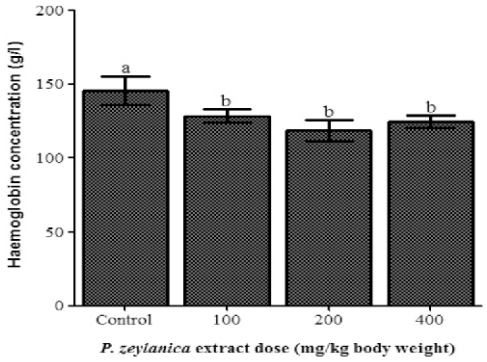

Figure 2: Effect of ethylacetate extract of *P. zeylanica* on haemoglobin concentration. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

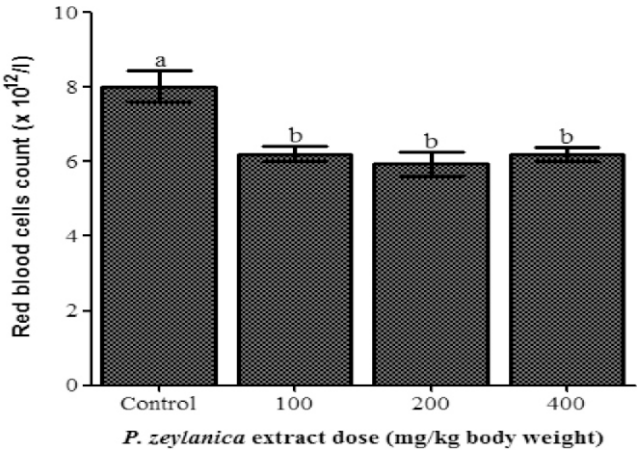

Figure 3: Effect of ethylacetate extract of *P. zeylanica* on red blood cells count. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

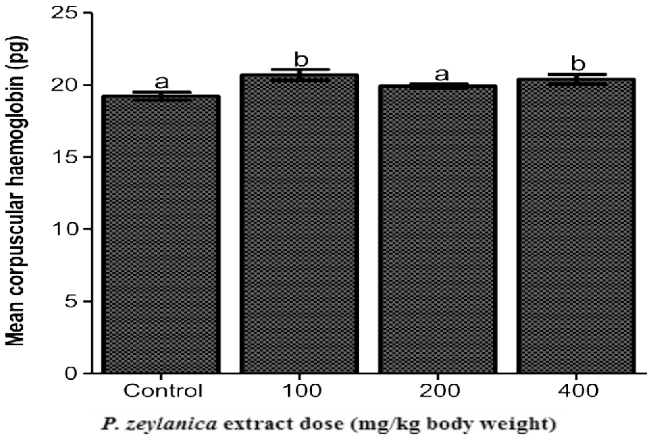

Figure 6: Effect of ethylacetate extract of *P. zeylanica* on mean corpuscular haemoglobin. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

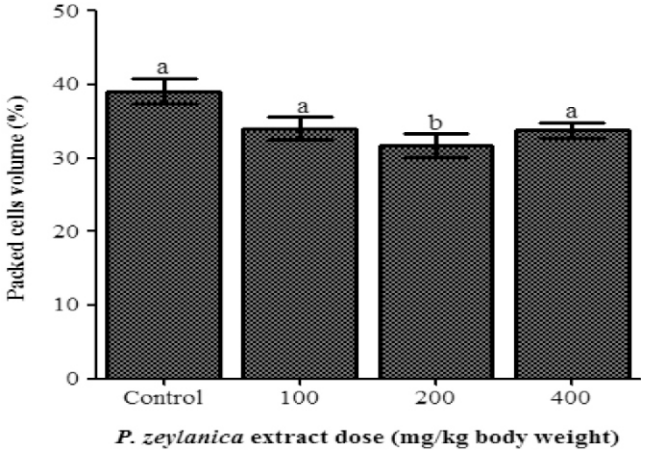

Figure 4: Effect of ethylacetate extract of *P. zeylanica* on packed cells volume. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

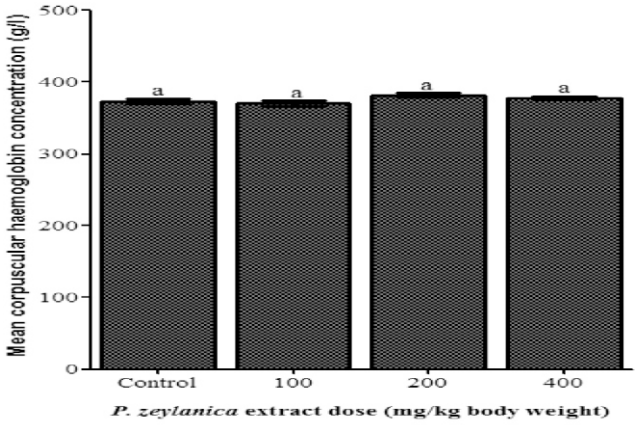

Figure 7: Effect of ethylacetate extract of *P. zeylanica* on mean corpuscular haemoglobin concentration. Each bar represents the mean±SEM of 8 rats. Bars with the same alphabet are not significantly different from each other *(p>0.05)*.

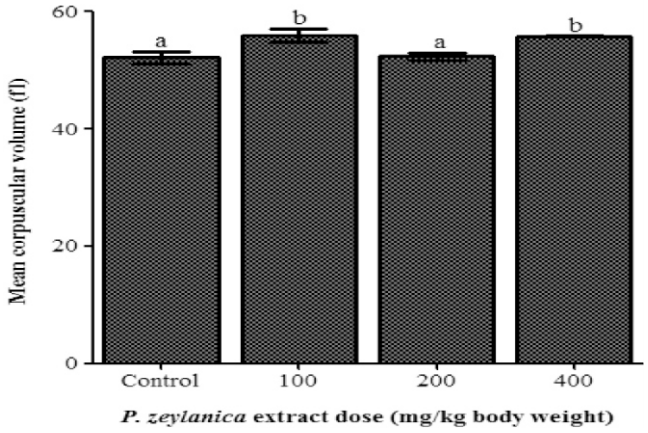

Figure 5: Effect of ethylacetate extract of *P. zeylanica* on mean corpuscular volume. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

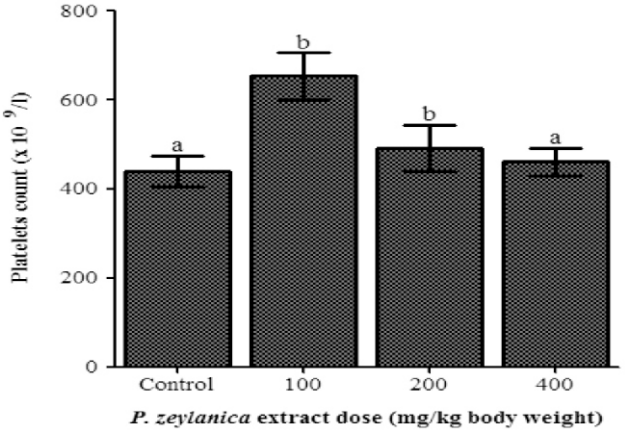

Figure 8: Effect of ethylacetate extract of *P. zeylanica* on platelets counts. Each bar represents the mean±SEM of 8 rats. Bars with different alphabets are significantly different from each other at $p<0.05$.

DISCUSSION

In acute toxicity study, no mortality was noticed up to 20,000 mg/kg body weight by the oral route whereas, 50% mortality was noticed in 100 mg/kg body weight by the intraperitoneal route. Hence, the extract is safe for oral use and this could explain the safe use of the plant by local people in traditional management of various ailments.[22] The symptoms of toxicity observed in animals during intraperitoneal administration of extract in acute toxicity studies include restlessness, freezing, writhing, hyperactivity and convulsion which eventually resulted to death. These symptoms also agreed with earlier scientific reports of Bruton and Pye.[23] Oral administration pre-supposes that the extract passed through the digestive tract. There is possibility that digestion would have modified or slow down absorption of the active constituents leading to non-toxic but active and efficacious principles. Administration through the intraperitoneal route no doubt has bypassed the digestive tracts with a higher possibility of preserving the active principles, hence the observed toxicity.

The vital function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoeitic system unique as a target organ.[24] Accordingly, it ranks with liver and kidney as one of the most important considerations in the risk assessment of potential environmental toxicants or xenobiotics. The various blood cells (erythrocytes, leucocytes, and platelets) are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological states including hemolytic anemia or suppressive inflammation.

The haematological effect of ethylacetate extract of P. zeylanica led to a significant increase in white blood cells count (WBC) and platelets (PLT), slight increase in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) while the red blood cells count (RBC), haemoglobin level (HB) and packed cell volume (PCV) decreased significantly in treated rats compared with the control. The increase in white blood cells count (WBC) may be due to its production from the bone marrow or reduced margination in blood vessels. Increase in the white blood cells count (WBC) could also be as a result of immune response to the inflammation of the intestines. The reduction in the values of red blood cells count (RBC) and its associated parameters, haemoglobin level (HB) and packed cell volume (PCV) could be as a result of suppressive action of extract on production of red blood cells (erythropoiesis).

Since mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) relate to individual red blood cells while haemoglobin (HB), red blood cells (RBC) and packed cells volume (PCV) are associated with total population of red blood cells,[25] alteration in the values of these indices is likely to affect the oxygen-carrying capacity of each of the red blood cells as well as its total population. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are important in diagnosis of anaemia in most animals [26]. Elevation of these indices implies the extract could cause megaloblastic changes in the marrow and elevation of platelets (PLT) also implies that the extract may have stimulatory effect on thrombopoietin.[27] Platelets (PLT) when present in sufficient size, number and function are involved in the process of normal coagulation of the blood but excess of it may also cause thromboembolism.[28]

CONCLUSION

Oral administration of very high doses of ethylacetate root extract of *P. zeylanica* showed no toxic effect in mice whereas the intraperitoneal administration of lower doses resulted into early behavioural changes and death of mice within 24-48 hours of the experiment. There was significant increase observed in white blood cells while significant reduction was noticed in haemoglobin and red blood cells of orally treated rats after 28 days. Decrease in red cell indices may indicate that the extract suppresses replication of erythrocytes. Whereas at high dose, the extract increases the white cell and platelet counts which may be a reactive effect due to stress on the marrow thus releasing more cells to the circulation. Therefore, the extract may not be useful in treating anaemia.

Conflict of Interest

We declare no conflict of interest in this study.

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