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EVALUATION OF SAFETY OF AQUEOUS, METHANOL AND DICHLOROMETHANE CRUDE EXTRACTS OF THE KENYAN *PHYSALIS PERUVIANA L* (CAPE GOOSEBERRY) IN MALE WISTAR ALBINO RATS

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ABSTRACT

The current study was undertaken with the aim of establishing the safety profile of different parts of *Physalis peruviana* L extracts in male wistar albino rats. The biochemical parameters were determined using Humanlyser 2000, a controlled photometer, while the haematological parameters were analyzed using HumanCount 5 L automated lazer haematology analyzer. Results on haematological parameters revealed that after 28 days of administration of Dichloromethane *Physalis peruviana* leaf and Dichloromethane *Physalis peruviana* stem crude extracts to rats at 500mg/kg /day, there were significant increase ($P<0.05$) in Red blood cell counts compared to the negative control group; there were also significant increases in the Haemoglobin concentration and Lympocytes counts in dichloromethane *P. peruviana* extracts-treated rats compared to controls. However, there was a significant decrease ($P<0.05$) in Neutrophils from dichloromethane *P. peruviana* as compared to the negative control group. The effects of extracts on biochemical parameters after treatments for 28 days revealed significant differences ($P<0.05$) in the decrease on Alanine transaminase and Aspartate aminotransaminase (AST) from dichloromethane *P. peruviana* stem and leaves treated groups compared to the negative control group. The extracts had no significant differences ($P>0.05$) on serum Alkaline phosphatase, total protein and total bilirubin. Peripheral blood films revealed marked Polychromasia in aqueous *Physalis peruviana* roots and acanthocytes in aqueous *Physalis peruviana* stem treated groups compared to the negative control. It can be concluded that extracts of *P. peruviana* at the doses tested had mild toxic effects and also lead to boosting of immunity in body systems.

KEYWORDS: *Physalis peruviana* L, Toxicity, Rats.

INTRODUCTION

Since ancient times medicinal and herbal plants have been used for treatment of infectious diseases due to presence of curative agents.[1] Natural products from plant extracts either in form of pure compounds or normalized extracts have unlimited opportunities for drug discoveries because of their unmatched chemical diversity.[2] The presence of valuable bioactive phytochemicals in plants is important in medicine as they play a part as chemical product alternatives.[3] In Kenya, as in many African countries, plants have been in use in traditional medicine for treatment of many infectious diseases.[4] According to a study carried out by Kimang'a *et al.*[5], it can be deduced that three quarters of world population relies on herbal and traditional health care. Further, according to Saenphet *et al.*[6], many people believe that use of biological agents is safe without knowing that lack of their dosage standardization may lead to toxicity.

Physalis peruviana L belongs to solanaceae family. Common English names for the plant include Cape gooseberry, Golden berry, Husk cherry, Peruvian Ground cherry and Poha berry. The plant is described as, a shrub, erect in habitat and bearing branches arranged in an alternate manner, bears pubescent, slightly-dentate heart-

shaped leaves. The flowers are described as yellow in colour, pendulous in nature and have corollas marked by purple or purplish-brown spots. [7]

In Kagera region, north western Tanzania, fruit juice of *P. peruviana* is used for the treatment of malaria.[8] In addition, the aqueous and 12% ethanol fruit extracts were shown to demonstrate antidiabetes and antihypertension properties *in vitro*.[9] *P.peruviana* boiled leaf concoction is taken orally to treat asthma.[10] The aqueous leaf extracts were found to be effective against three gram negative bacteria (*Escherichia coli, Proteus vulgaris, Seratia marsescens*) and three gram positive bacteria (*Bacillus subtilis, Micrococcus kristinae, Staphylococcus aureus*).[11] *P.peruviana* calyxes has been used in folklore medicine as an anticancer, antimycobacteria, antipyretic, diuretic, immunomodulator and as an anti-inflammatory agent. [12]

The purpose of this study was to evaluate the safety profile of *P.peruviana* L extracts from roots, stem and leaves extracted using water, methanol or dichloromethane on male wistar albino rats.

MATERIALS AND METHODS

Plant materials

The whole plant materials were collected from Nyeri County [0°25`0``South, 36°57`0``East] located in Central Kenya in the month of February, 2013. The natural habitats of Nyeri County have good reserves of *Physalis peruviana* L hence the choice of the location. The identification of the plant material was carried out by the National Museums of Kenya Botanists and a voucher specimen number EAH001PK retained. The plant parts; leaves roots and stems were separated, dried under shade and milled in a hammer mill fitted with a sieve of 0.5 mm size.

Plate. 1: Arial parts of fruity *Physalis peruviana* L plant.

Botanical classification: According to Sharma *et al*.[13], *Physalis peruviana* L can be botanically classified as to belong to the Plantae Kingdom, the order of solanales, the Family of solanaceae, Subfamily of solanoideae, Tribe of physaleae, Sub tribe of physalinae and the species of *Physalis peruviana* L.

Preparation of plant extracts: The extraction of the plant materials was carried out using the methods employed by Ubulom *et al*.[14] Pulverished leaves, stem and roots were separately soaked in distilled water, methanol and dichloromethane, for 72 h with stirring regular at regular intervals. Whatman No. 1 filter paper[11], was used to filter the extracts repeatedly before the aqueous filtrates were freeze dried, while the methanolic and dichloromethane extracts were concentrated under vacuum in a rotary evaporator. The extracts were coded for identification as follows: APPR = aqueous extracts of *Physalis peruviana* L root; APPS = aqueous extracts of *Physalis peruviana* L stem; MPPR = methanolic extracts of *Physalis peruviana* L root; MPPS = methanolic extracts of *Physalis peruviana* L stem; DPPS = dichloromethane extracts of *Physalis peruviana* L stem; DPPL = dichloromethane extracts of *Physalis peruviana* L leaves. The extracts were then weighed and stored in sealed containers at 4^0C until use.

Sources of chemicals: Methanol and dichloromethane were procured from Fisher Scientific, UK, ltd, while standard biochemical assay kits were procured from Roche diagnostics ltd, United Kingdom. All other chemicals used in this study were analytical grade.

Experimental animals: Male albino Wistar rats aged 8 to 16 weeks and weighing 150 to 200 g were used in this study. The animals were obtained from the animal house of Jomo Kenyatta University of Agriculture and Technology, Kenya.They were housed in 15-cm x 21-cm x 29-cm transparent cages bedded with wood chips and equipped with continuous – flow nipple watering bottles and fed with standard rat feed (rat pellets UNGA feeds Kenya ltd) and water ad-libitum. They were maintained under standard laboratory conditions (temperature 25 ± 2^0C with dark/light cycle 12: 12h). The study was approved by the Ethical Review Committee of Kenya Medical Research Institute (KEMRI), Kenya.

Experimental Design

Acute Toxicity Test

Oral acute toxicity of crude *Physalis peruviana* L extracts was carried out using Wistar male albino rats. A total of one hundred and five Wistar male albino rats were assigned into eighteen experimental groups and three control groups of 5 animals each and labeled with

permanent marker pen. Single doses of each of the six crude extracts, aqueous extracts of *Physalis peruviana* L root (APPR), aqueous extracts of *Physalis peruviana* L stem (APPS), methanolic extracts of *Physalis peruviana* L root (MPPR), methanolic extracts of *Physalis peruviana* L stem (MPPS), dichloromethane extracts of *Physalis peruviana* L stem (DPPS) and dichloromethane extracts of *Physalis peruviana* L leaves (DPPL) was administered to the experimental rat groups orally at 1000 mg/kg, 2000 mg/kg and 5000 mg/kg body weight by use of an oral gavage needle fixed onto a syringe; the control groups of rats were administered with physiological saline (PSG). The amount of the extracts and saline administered to the rats in milliliters was calculated according to guidelines on dosage calculation and stock solution preparation in experimental animal studies.[15] The doses administered in this experiment were slightly in modification of Bhattacharya method. [16] The animals groups were kept in separate cages and observations for general behavior or deaths taken within a period of three days. Any abnormal behavior or mortality was recorded till the end of the study.

Sub-Chronic Toxicity Test

Sub-chronic toxicity of *Physalis peruviana* L extracts was carried out using the method of Bhattacharya[16], with slight modification. A total of thirty five Wistar male albino rats were assigned to six experimental groups and one control group of 5 animals each and labeled with permanent marker. Body weights of all the rats were determined before administration of extracts or saline to experimental and control groups respectively. The experimental groups were orally treated with one tenth highest tolerable dose in acute toxicity study ($^{1}/10^{th}$ of 5000 mg/ kg body weight) using oral gavage needle; while the control group received saline through the same route for a period of 28 days consecutively. On day 29 after an overnight fasting, each rats' body weight was determined and consequently anaesthetized with diethyl ether. Blood samples from the right ventricle[17] was collected for haematological parameters in tubes incorporated with ethylene diamine tetra-acetic acid (EDTA), while that for biochemical parameters was collected into heparinized tubes. The animals were sacrificed by withdrawing large amounts of blood, the organs removed and their weights determined.

Haematological assays

The anticoagulated blood was analysed for haematological parameters at the Kenya Wildlife Veterinary laboratories of Kenya using the HumanCount 5 L, an automated lazer hematology analyzer from Chem labs Nairobi Kenya. The haematological parameters that were analyzed included White blood cells (WBC), Red blood cells (RBC), Hemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular Hemoglobin concentration (MCHC), Mean corpuscular Hemoglobin (MCH), Mean cell distribution width (MCW), Platelets (PLTs) and differential White blood cell counts for Neutrophils percent (NEUT %), Lymphocyte percent (LYMP %), Monocyte percent (MONO %), Eosinophil percent (EOS %) and Basophil percent (BASO %). Consequently peripheral blood films (PBFs) were prepared and stained with wrights stain for cell morphological change determination.

Biochemical assays: Biochemical assays for evaluation of toxic effects at organ levels were investigated by using standard assay kits (Roche diagnostics ltd, United Kingdom). Aspirated sera was injected into Humanlyser 2000, a controlled semi-automatic microprocessor controlled photometer from Chem labs Nairobi, Kenya, in the laboratories of the Kenya Wildlife Veterinary laboratories. The biochemical parameters that were analyzed included; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), serum alkaline phosphatase (SALP), Total protein, serum cholesterol and Total bilirubin.

Statistical analysis: The tool used to enter and capture data was Microsoft Excel. The primary data was analyzed and presented ± Standard error of the mean. The data was statistically analyzed using one way analysis of variance (ANOVA) Vassarstas for independent samples and level of significant in test values versus control set at $p < 0.05$.

RESULTS

Acute Toxicity Test: Acute toxicity study involved oral administration of *Physalis peruviana* L extracts (APPR, APPS, MPPR, DPPS, DPPL) to male Wistar albino rats. No deaths were noticed 48 hours after administration of each extract to a group of 5 male Wistar albino rats at doses of 1000, 2000 and 5000 mg/kg body weight. There were no adverse clinical signs of toxicity, such as tremor, irritability, diarrhea, paralysis, skin and fur changes, convulsion or drowsiness noticed during the 72 – hour period on the experimental and control group.

Sub-Chronic Toxicity Test

Effects of *Physalis peruviana* L extracts on body and organ weights: During and after the 28 days experimental period of administering 500 mg/kg body weight with the extracts (APPR, APPS, MPPR, DPPS, DPPL) or the respective volume of saline to control group, no deaths or toxicity signs were noted in both experimental and control group. There was however a significant decrease in final body weight ($p<0.05$) in the groups treated with MPPR, DPPS and DPPL compared to the control group (Table 1). These differences in mean body weight of crude extract treated rats/compared to control rats ranged from 4.8 to 8.4 % decrease.

Table. 1: Effects of *Physalis peruviana* L extracts on body and organ weights of male Wistar albino rats. The values are expressed as mean weights ± standard error of the mean (n=5).

Parameter	Control	APPR	APPS	MPPR	MPPS	DPPS	DPPL
Initial bwt (g)	176±8	195.2±2.3	172±9	167.2±6	190±6.3	189.8±4.8	179.8±8
Final bwt (g)	194±7.48	207.6±4	193.6±6	159.2±7*	183.4±5.4	177.9±3.1*	164.8±6.4*
Final Heart wt (g)	0.7±0.08	0.9±0.07	0.5±0.05	0.6±0.05	0.7±0.01	0.6±0.05	0.562±0.06
Final lung wt (g)	1.7±0.19	2.2±0.81	1.6±0.11	1.5±0.13	2.1±0.27	1.3±0.07	1.7±0.43
Final Liver wt (g)	8.2±0.63	9.9±0.91	6.8±0.76	6.5±0.92	7.3±0.69	5.3±0.17	4.6±0.59
Final Kidney wt (g)	1.7±0.08	1.8±0.13	1.5±0.17	1.5±0.18	1.8±0.07	1.06±0.29	1.48±0.08
Final Pancreas wt (g)	0.33±0.03	0.28±0.04	0.25±0.05	0.29±0.02	0.29±0.04	0.28±0.03	0.26±0.03

Key: APPR = aqueous extracts of *P. peruviana* L roots; APPS = aqueous extracts of *P.peruviana* L stem; MPPR = methanolic extracts of *P. peruviana* L roots; MPPS = methanolic extracts of *P. peruviana* L stem; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPL = dichloromethane extracts of *P. peruviana* L leaves; Values with superscripts in extracts administered groups were significantly different from control groups (*denotes that P<0.05).

Effects of *Physalis peruviana* L extracts on biochemical parameters: Results on biochemical parameters are as shown in Table 2. Twenty eight (28) days of treatment of the rats with 500 mg/kg (APPR, APPS, MPPR, DPPS, DPPL) body weight revealed significant differences (P<0.05) in decrease on Alanine amino transaminase (ALT) and Aspartate aminotransferase (AST) from DPPS and DPPL treated groups compared to the negative control group. However the extracts had no significant serum alkaline phosphatase (SALP), total serum protein and bilirubin levels (P>0.05).

Table. 2. Effects of *Physalis peruviana* L extracts on biochemical parameters in male Wistar albino rats. The values are expressed as mean ± standard error of the mean (n=5).

Parameter	Control	APPR	APPS	MPPR	MPPS	DPPS	DPPL
ALT (U/L)	25.3±1.85	29.3±2.5	27.9±3.27	30.6±4.54	29.5±4.77	16.7±0.9*	15.6±1.01*
AST (U/L)	127.0±4.06	132.6±14.8	141.3±13.7	122.4±3.33	145.3±14.06	83.6±7.8*	95.7±3.9*
SALP (U/L)	89.5±2.72	96.8±29.70	105.4±36.8	163.1±62.8	114±17.5	100.4±5.3	103.4±5.8
Total Protein (g/dl)	7.2±0.44	7.8±0.78	6.1±0.42	8.1±0.88	6.6±0.64	7.4±0.33	7.9±0.41
Cholesterol (mg/dl)	111.2±9.07	67.6±2.8*	65.9±5.56*	62.22±6.03*	61.6±5.88*	63±9.03*	68.1±3.93*
Total Ilirubin (µlmol/L)	0.34±0.07	0.24±0.07	0.3±0.13	0.3±0.13	0.32±0.08	0.48±0.15	0.32±0.08

Key: APPR = aqueous extracts of *P. peruviana* L roots; APPS = aqueous extracts of *P.peruviana* L stem; MPPR = methanolic extracts of *P. peruviana* L roots; MPPS = methanolic extracts of *P. peruviana* L stem; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPL = dichloromethane extracts of *P. peruviana* L leaves; Values with superscripts in extracts administered groups were significantly different from control groups (* denotes that P<0.05).

Effects of *Physalis peruviana* L extracts on haematological parameters: Treatment of rats for twenty eight (28) days with 500 mg/kg body weight extracts (APPR, APPS, MPPR, DPPS, DPPL) revealed significant increase in the following parameters with (p<0.05): RBC in DPPS and DPPL; HB and lymphocytes in DPPL as compared to controls. However, a significant decrease (p<0.05) was noted in Neutrophils in DPPL as compared to the negative control group as shown in Table 2.

Table 3. Effect of *Physalis peruviana* L extracts on haematological parameters in male Wistar albino rats. The values are expressed as mean ± standard error of the mean (n=5).

Parameter	Control	APPR	APPS	MPPR	MPPS	DPPS	DPPL
WBC (x 10^3 µl)	5.4±0.71	20.6±8.81	4.74±0.39	5.4±0.77	3.8±0.48	7.84±0.5*	7.92±0.7*
RBC (x 10^6 µl)	7.4±0.73	7.02±0.46	8.7±0.31	8.5±0.38	8.6±1.32	9.4±0.20*	9.9±0.41*
HB (g/dl)	13.7±0.47	15±0.52	14.2±0.26	13.7±0.36	14.8±0.32	13.8±0.14	15.2±0.43*
PCV (%)	44.8±1.32	40.2±2.90	46.4±1.64	45.2±1.66	47.9±1.22	46.1±0.50	49.4±1.56
MCV (fl)	57.2±1.66	59.0±1.74	52.4±1.28	55.6±1.2	56.6±1.7	55±1	52.46±0.52
MCHC (g/dl)	43.9±6.61	34.7±1.18	31.6±0.98	30.2±0.57	31.2±0.55	29.5±0.46	30.4±1.05
MCH (pg)	23.8±3.70	20.7±1.12	16.6±0.81	30.2±0.57	27.4±3.33	20.96±6.4	15.3±0.93
RDW (%)	18.6±1.18	20±0.61	17.9±0.43	19.04±0.92	15.9±0.94	19.5±0.30	18.1±0.31
PLTs (x 10^3 µl)	826.4±138.5	1055.2±58.43	947.4±37.7	976.6±126±.99	1117.4±40.93	1004.2±44.51	891.2±34.18
NEUT (%)	28.8±7.86	14.4±1.73	24.4±4.21	18.8±3.13	13.2±1.74	22.6±2.08	8.6±1.60*

LYMP (%)	61.6±6.73	76.2±2.28	67.6±4.11	75.2±3.44	77.2±2.57	67±1.26	82.4±2.03*
MONO (%)	4.4±0.4	7.2±2.87	5.6±0.97	4.4±1.32	4.8±1.49	4.8±1.35	5.2±2.33
EOS (%)	5.2±1.49	2.2±1.49	2.4±0.74	1.6±1.16	2.4±1.6	6±1.78	3.2±1.95
BASO (%)	0	0	0	0	0	0	0.6±0.40

Key: APPR = aqueous extracts of *P. peruviana* L roots; APPS = aqueous extracts of *P.peruviana* L stem; MPPR = methanolic extracts of *P. peruviana* L roots; MPPS = methanolic extracts of *P. peruviana* L stem; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPL = dichloromethane extracts of *P. peruviana* L leaves; Values with superscripts in extracts administered groups were significantly different from control groups (* denotes that P<0.05).

Effects of *Physalis peruviana* L extracts on cell morphological changes: Peripheral blood films (PBFs) from groups fed with 500 mg/kg body weight extracts (APPR, APPS, MPPR, DPPS, DPPL) for the twenty eight (28) day period revealed some morphological changes including marked polychromasia in APPR and acanthocytes in APPS as compared to control group showing normal morphological features (‘‘fig’’ 1, 2 and 3).

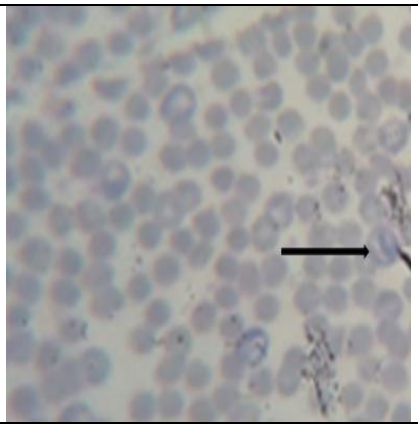

Figure. 1: the arrow points to a polychromatic RBC (magnification X 100).

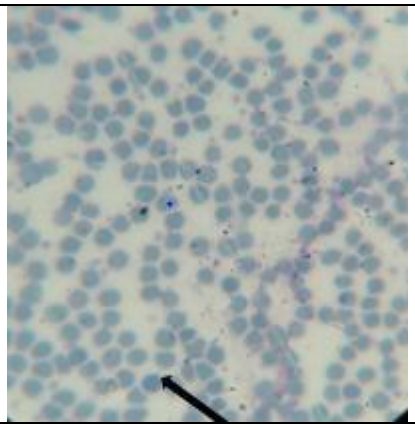

Figure. 2: The arrow points to a normal RBC (magnification X 100).

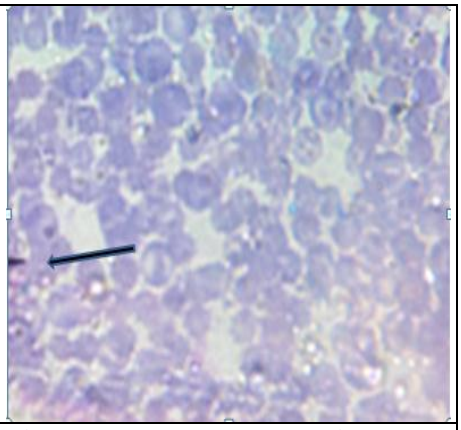

Figure. 3: arrow points to Acanthocyte (magnification X 100).

DISCUSSION

It has been documented that many studies of *Physalis peruviana* L were conducted on the fruit[18], and in another study the fruit extracts were found to be of lesser activity to microbes compared to other extracts.[1] Therefore the study to evaluate safety profile on *Physalis peruviana* L extracts involved other parts (APPR, APPS, MPPR, MPPS, DPPS and DPPL), using male wistar albino rats.

Contrary to the belief that use of natural products is safe, only a handful of Scientific papers have reported toxicity derived from plant materials both in animals and non target microorganisms.[6] It is necessary to determine safety profile of plant extracts due to the complexity and natural biological variations emanating from them.[19] In this study the plant materials were collected from Nyeri County [$0^{o}25`0``$South, $36^{o}57`0``$East] located in Central Kenya. From previous studies, environmental factors, geographical origin, part of the plant used, method of extraction and preparation of extracts have had effects on phytochemical contents and marker compounds.[5,19]

From acute toxicity study, no signs of toxicity or deaths were revealed on administration of 1000, 2000 or 5000 mg/kg body weight from the extracts (APPR, APPS, MPPR, MPPS, DPPS and DPPL) after observing them for a period of 48 hours.

The effects of *Physalis peruviana* L extracts on body and organ weights are shown on Table 1. Administration of 500 mg/kg body weight in sub-chronic toxicity and observing the animals for 28 days revealed no signs of toxicity or deaths compared to the control group.

The results revealed a significant decrease in final body weight to male wistar albino rats treated with, MPPR, DPPS and DPPS as compared to the control group. An increase or decrease in body weight could have been associated with physiological changes such as liver function, poor protein and amino acid absorption and hormonal changes.[17] Further it has been postulated that stress caused by oral gavage on the digestive system may result in animals having difficulties in feeding resulting in weight loss.[17] There were no significant differences in organ weights in experimental animals as compared to the control.

Evaluation of biochemical parameters is an invaluable investigation in that, many reports related to kidney and liver toxicity are associated with therapeutic products.[20] In the present study, biochemical serum parameters analyzed after treating male wistar albino rats are

presented in Table 2. Significant decreases in serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) both in DPPS and DPPL extracts ($P<0.05$), as compared to the negative control group were observed. The significant decrease in ALT and AST can arise due to liver disturbances.[18] These results are in contrast with those carried by Perk *et al*. [18], in which *Physalis peruviana* juice had no significant differences in ALT and AST levels ($P>0.05$), compared to the control. The experimental animals revealed a significant decrease with serum cholesterol ($P<0.05$). From a previous study that analyzed biochemical parameters in *Physalis peruviana* extracts[1], tannins were detected which are thought to prevent oxidation of LDL cholesterol, consequently lowering body fat and prevent heart disease.[21] A decrease in serum cholesterol can also occur as a result of lowered intestinal absorption of cholesterol emanating from increased excretion of neutral lipids in faeces.[20] There were no significant differences in serum alkaline phosphatase (SALP), total serum protein and total serum bilirubin ($P>0.05$).

The experimental and control groups' blood was used for a full blood count determination. Evaluation of haematological parameters assists in determining risks of hematopoietic alterations that may arise from toxic effects from plant phytochemicals requiring necessary actions before being applied to humans.[22]

Treatment of rats with DPPS and DPPL extracts revealed significant increases in white blood cell (WBC) and red blood cell (RBC) indices as compared to the negative control group. An increase in WBC and RBC indices could emanate from stimulation of hematopoetic sites making them undergo consequential division and mutational changes resulting in leukopoiesis and erythropoiesis.[22] A significant ($P<0.05$) increase in levels of heamoglobin (HB), lymphocytes (LYMP) and a decrease in Neutrophils (NEUT) ($P<0.05$) was revealed in DPPL extract treated group as compared to the negative control group. An increase in HB and LYMP can also be due to the erythropoetic and leukopoesis processes. Peripheral blood films (PBFs) from rats treated with extracts revealed some morphological changes such as Polychromasia and acanthocytes in APPR and APPS as compared to the control group (figs. 1 and 3 respectively. Polychromasia also known as polychromatophilia is a disorder where there is an abnormally high number of immature red blood cells in the bloodstream as a result of being prematualy released from the bone marrow. Acanthocytes are spur or speculated cells seen in patients with liver disease, postsplenectomy and abetalipoproteinemia.

CONCLUSIONS

From this study, it can be concluded that extracts from *P. peruviana* L at the doses tested had mild toxic effects and are also liable to boosting immunity in body systems.

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AUTHORS DISCLOSURE STATEMENT

We attest that the submitted work represents our contributions, have not been copied or plagiarized in whole or part from other works. No conflict of interests exists.

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