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Toxicological and Biosafety Evaluation of Polyherbal Tea Formulation

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Abstract

The safety evaluation of herbal formulations is essential for establishing their suitability for therapeutic development and use. This study assessed the acute and subacute toxicity profiles of a polyherbal tea formulated from *Ageratum conyzoides*, *Azanza garckeana*, *Anthocleista djalonensis*, *Allium sativum*, and *Zingiber officinale*. Acute toxicity was evaluated in mice using a modified Lorke's method, whereas subacute toxicity was assessed in albino rats administered the formulation at doses of 0.25, 0.5, and 1.0 g/kg according to a standard protocol. No mortality occurred at 10 g/kg; therefore, the median lethal dose could not be determined. The formulation significantly increased body weight at all tested doses compared with the control group ($p < .05$). It reduced the spleen-to-body weight ratio ($p < .05$) but produced no significant changes in the relative weights of the heart, liver, kidneys, or lungs ($p > .05$). At 0.5 g/kg, haemoglobin concentration and platelet count decreased significantly ($p < .05$), whereas the remaining haematological parameters were unchanged. Alkaline phosphatase, creatinine, urea, total cholesterol, and triglyceride levels decreased significantly at all tested doses ($p < .05$). Aspartate aminotransferase and albumin levels decreased at 0.5 g/kg, while alanine aminotransferase decreased at 0.25 and 0.5 g/kg ($p < .05$); all other biochemical parameters remained

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stable. Histopathological examination revealed normal architecture of the kidneys, heart, lungs, and spleen, although hepatic steatosis was observed. Overall, the formulation demonstrated a high acute tolerance and limited subacute alterations at the tested doses. However, the reductions in haemoglobin and platelet counts and the presence of hepatic steatosis indicate that its biosafety profile should be interpreted cautiously. These findings provide preliminary evidence for the toxicological characterisation of the polyherbal tea and support further dose-dependent and long-term safety investigations.

Keywords: Acute Toxicity; Biosafety Evaluation; Hepatic Steatosis; Polyherbal Tea; Subacute Toxicity

INTRODUCTION

Herbal medicine—'phytomedicine'—is the oldest form of medicine. Early humans appreciated the variety of plants and animals. Health issues have forced humans to seek remedies since the beginning of time (Loha *et al.*, 2019). Herbal medicine is used for primary health care by 80% of the world, according to the WHO. Herbal medicine is used by 80% of Asian and African populations for primary health care (WHO, 2023). Herb use is influenced by gender, age, education, socioeconomic status, ethnicity, and finances. NAFDAC and other organisations regulate herbal medicines (Iyiola and Adegoke, 2023; Ogah and Orgah, 2015; Osuide, 2002). Herbs are usually taken as herbal tea or a polyherbal tea of plant extracts for synergistic therapeutic effects (WHO, 2023). Plant extracts contain active phytochemicals that have shown efficacy in research. The WHO endorses herbal medicine if it is effective and safe (WHO, 2023). Industrial and scientific communities in Nigeria are interested in herbal medicine.

The Alliaceae family includes garlic (*Allium sativum* L.) (Gomes et al., 2021; Londhe, 2011). The plant is medicinal because it can prevent cardiovascular disease, regulate blood pressure, fight bacterial, viral, fungal, and parasitic infections, boost immunity, and fight cancer. The plant reduces glycaemia and high blood cholesterol (Barbu et al., 2023; Goncagul and Ayaz, 2010; Ayaz and Alpsoy, 2007). It helps with indigestion, respiratory and urinary tract infections, cardiovascular conditions, and fevers and is carminative, sedative, aphrodisiac, and diuretic. Ginger (*Zingiber officinale*) is a flowering plant in the kingdom Plantae, order Zingiberales, family Zingiberaceae, genus Zingiber, and species Z.

officinale. It relieves cold symptoms like influenza, cephalalgia, dysmenorrhoea, arthritis, colic, diarrhoea, cardiovascular issues, motion sickness, early pregnancy nausea, and chemotherapy-induced nausea. It can also treat mild cardiovascular disease, osteoarthritis, and gastrointestinal issues. It is antioxidant, anti-inflammatory, antimicrobial, anti-lipidemic, and antidiarrheal (Vasala, 2012; Khan et al., 2016; Yousfi, 2021; Auta, 2011).

The Kingdom Plantae, Division Angiosperms (a tracheophyte), Order Asterales, Family Asteraceae, Genus Ageratum, and Species A. conyzoides (Kaur et al., 2023) include "billygoat-weed", "goatweed", "whiteweed", and "mentrast". Traditional cultures treat wounds, diarrhoea, dysentery, and skin infections with ageratum conyzoides. It's a mouthwash, it's antibacterial, and it's a wound dressing (Panda, 2018). Whole plant extracts diluted in water reduced arthritis pain and improved mobility. It also treats pneumonia, dyspepsia, rheumatism, fever, colic, and burn wounds. *Azanza garckeana,* called 'Goron Tula' in Hausa (kola of Tula) (Yusuf *et al*., 2020; Michael, 2015). It has anti-inflammatory, analgesic, antimicrobial, antioxidant, fecundity, and reproductive properties (Bukar et al., 2020; Yusuf, 2020). This herb reduces the effects of coughs, thoracic pain, infertility, irregular menstruation, sexually transmitted infections, and hepatic dysfunctions by increasing insulin and glucose production. Southwest Nigerian Yoruba calls *Anthocleista djalonensis* "Ewe Shapo". The Hausa, Igbo, and Yoruba call Anthocleista djalonensis 'Kwarii', 'Okpokolo', and 'Sapo', respectively. African traditional medicine treats diabetes, hypertension, malaria, typhoid fever, obesity, diarrhoea, dysentery, hyperprolactinaemia, ulcerative colitis, jaundice, asthma, haemorrhoids, hernias, cancer, wounds, chest pain, inflammation, rheumatism, sexually transmitted diseases (gonorrhoea and syphilis), and infertility with Anthocleista djalonensis. Common uses include anthelmintic, diuretic, and contraceptive (Kagbo and Simon, 2015; Akpan, 2012). Leaf, bark, or root infusions are taken in water for laxative and calming effects (Adebayo and Olamide, 2022; Taiwe et al., 2017). This study will assess the biosafety of a polyherbal tea containing *Ageratum conyzoides, Azanza garckeana, Anthocleista djalonensis, Allium sativum, and Zingiber officinale*.

MATERIALS AND METHODS

Plant Collection

Allium sativum (garlic), and *Zingiber officinale* (ginger) were purchased from Kurmi market within Kano municipal local government area, Kano state, Nigeria. *Azanza*

garckeana (Goron tula), was purchased at Tula village in the Kaltungo Local Government Area, Gombe state. *Ageratum conyzoides* (goat weed) and *Anthocleista djalonensis* leaves were gotten from Ikpoba - Okha Local Government Area in Edo State, Nigeria.

Preparation of Plant Materials/Polyherbal Tea Formulation

Garlic and ginger were washed with clean water and chopped into smaller bits. The gorontula fruits were washed and the seed were removed from within the hard shell cover. The chopp Garlic and ginger were rinsed with clean water and diced into smaller pieces. The gorontula fruits were cleansed, and the seeds were extracted from the brittle shells. The minced garlic, ginger, and gorontula seeds were desiccated utilising a dehydrator. The leaves of goatweed and Anthocleista adjalonensis were rinsed with purified water and air-dried. Following dehydration, the goat weed, Anthocleista djalonensis, garlic, ginger, and goron-tula seeds were individually ground into powder using an industrial blender (KENWOOD Model: KCB239K) and subsequently stored in appropriately labelled, clean, dry containers. The polyherbal tea was formulated with Ageratum conyzoides. Anthocleista djalonensis, Allium sativum, Zingiber officinale, and Azanza garckeana in the ratio of (1:1:1:1:1/3), respectively. The herbal tea was designed so that each tea bag contained 200 mg of each powdered botanical ingredient.

ed garlic, ginger and the gorontula seeds were dehydrated using a dehydrator. The goat weed and *Anthocleist adjalonensis* leaves were washed with clean water and air-dried. After dehydration, the goat weed, *Anthocleista djalonensis*, garlic, ginger and goron-tula seeds were blended to powder separately using the industrial blender (KENWOOD Model: KCB239K) and put in a well labelled dried clean container respectively. The polyherbal tea was prepared using the formulation of *Ageratum conyzoides* , *Anthocleista djalonensis, Allium sativum, Zingiber officinale* and *Azanza garckeana* in the proportion ratio of (1:1:1:1:1/3) respectively. The herbal tea was formulated in such a way that a tea bag contained 200 mg of each powdered plant material.

Polyherbal Tea Extraction

The polyherbal tea was extracted based on the methodologies of Uwaya and Idu (2022) and Owolabi *et al.* (2008). 657 g of the formulated polyherbal tea was measured into a 20 L transparent container, to which 12.25 L of distilled water was added for aqueous extraction. The mixture was agitated with a stirrer and permitted to macerate for 72 hours, after which the solution was filtered through a strainer into a lidded storage container. The

aqueous extract was concentrated using a water bath, resulting in a 29% extract concentrate. The weight of the concentrate was determined using the weighing-by-difference technique. The aqueous extract concentrate was preserved in a properly labelled clean plastic container in a refrigerator at 4°C.

Experimental Animals

Healthy male albino mice, weighing 20–30 g, and adult rats, weighing 73–96 g, were acquired from the animal facility at the University of Ibadan, Oyo State. The mice and rats were housed separately in the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, and underwent a two-week acclimatisation period under standard laboratory conditions with a 12-hour light/dark cycle. The litter was replaced thrice weekly to maintain hygiene and optimise the animals' comfort. The subjects were provided with standard animal pellets and managed according to established protocols for laboratory animals (National Institute of Health, USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

Experimental Design

Acute Toxicological Assessment

Phase 1 and 11 acute toxicological assessments using mice.

The median lethal dose (LD50) was assessed using a modified Lorke's method, as per Ozolua *et al.* (2016). In the phase one (1) acute toxicity studies, nine (9) healthy mice, having undergone overnight fasting, were randomly divided into three groups of three mice each (n = 3) and assigned distinct identification numbers. Each group received dosages of 0.01, 0.1, and 1 g/kg of body weight, respectively. In phase 2, various mice were used and administered doses of 5, 7.5, and 10 g/kg of the extract to individual mice.

Following the oral administration of the polyherbal tea formulation, the animals were subjected to continuous observation for 1 hour and intermittent observation for 4 hours and subsequently monitored once every 24 hours for 14 days for any behavioural indications of toxicity or mortality (Uwaya and Idu, 2022; Loha et al., 2019).

Sub-Acute Toxicological Assessment using Healthy Albino Adult Male Rats

This study involved the assessment of twenty (20) healthy adult albino rats. The animals were fasted overnight and divided into four groups of five rats each (n = 5), comprising both males and females, as follows:

Group 1 was administered 2 ml/kg of distilled water.

Group 2 was administered 0.25 g/kg of a polyherbal tea extract.

Group 3 was administered 0.5 g/kg of a polyherbal tea extract.

Group 4 was administered 1.0 g/kg of a polyherbal-formulated tea.

The treatment was administered orally via an orogastric tube for 28 consecutive days. The animals' weights were recorded weekly. At the conclusion of the 28 days, the animals were humanely euthanised using chloroform anaesthesia, subsequently dissected through the abdominal region, and had blood extracted via the abdominal aorta. Blood was collected in EDTA containers for haematological analysis and lithium-heparin containers for biochemical analysis. The internal organs, comprising the heart, liver, kidneys, spleen, and lungs (essential organs), were excised and weighed, with each organ preserved in 10% formal saline for histological analysis. The blood in lithium containers was centrifuged at 2500 revolutions per minute (rpm) for 5 minutes, after which the serum supernatant was transferred to plain containers. Both whole blood and serum were subsequently stored in a refrigerator at -20°C.

Haematological Assay

The blood in the EDTA container was analysed using an automated haematological analyser, model PCE – 2100, JAPAN.

Biochemical Analysis

The biochemical parameters were analysed following the manufacturer's instructions (AGAPPE and RANDOX KITS). The procedures adhered to the guidelines established by Dickson and MacDonald (2022).

Histological Assessment

The fixed tissue specimens were dehydrated in a graded series of alcohol, treated with xylene, and embedded in paraffin wax before being sectioned with a microtome. Tissue sections (5 µm thick) were deparaffinised using xylene and subsequently hydrated in

alcohol prior to staining with haematoxylin and eosin. All sections were examined using a Leica® light microscope (Model DM500) at magnifications of x100 for lung tissue and x400 for heart, liver, spleen, and kidney tissues by a histopathologist who was unaware of the experimental groupings and protocols.

Statistical Analysis

The data were expressed as mean ± standard error of the mean (SEM), with 'n' denoting the number of mice in each experimental group. A one-way analysis of variance (ANOVA) was performed, succeeded by the Newman-Keuls post hoc test. Data analysis was conducted using GraphPad Prism software version 6 from the UK. A significance revealed substantial disparities between the analysed data.

RESULTS

Acute Toxicological Study of Aqueous Extract of Polyherbal Formulated Tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum* and *Zingiber officinale*).

The LD50 could not be ascertained because there was no death recorded at phases 1 and 2 of the study. The signs of toxicity demonstrated by the mice as a result of administration were paw licking,writhing reflex, stretching, drowsiness, tachypnea, restlessness, jerking **(Table1).**

Table1: The effect of acute toxicological study of polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum* and *Zingiber officinale).*

PHASE	DOSE (g/Kg)	ND/TA	TS OBSERVED
1	0.01 g/Kg	0/3	__
	0.1 g/Kg	0/3	__
	1.0 g/Kg	0/3	Paw licking, Stretching, Tachypnea,
11	5.0 g/Kg	0/3	Tachypnea, Drowsiness
	7.5 g/Kg	0/3	Stretching, Restlessness, Jerking
	10.0 g/Kg	0/3	Tachypnea, Writhing reflex

The animals were observed for 72 hours and then for additional 11 days after extract administration.

ND = Number of Death, TA = Total Number of Animals, TS = Toxicity Signs

In a Subacute Toxicity Study the poly-herbal tea formulation at doses of 0.25 g/kg, 0.5 g/kg, and 1 g/kg significantly enhanced the weights of albino rats during weeks 1, 2, 3, and 4, respectively, in comparison to the control group ($P < 0.05$). Illustration 1. The polyherbal tea formulation markedly reduced the spleen-to-body weight ratio compared to the control ($P < 0.05$) (Figure 5) and exhibited no impact on the heart, liver, kidneys, or lungs relative to body weight. The polyherbal tea formulation markedly reduced the spleen-to-body weight ratio compared to the control ($P < 0.05$) (Figures 2 to 4, 6). Haematological parameters indicated that the polyherbal tea formulation at 0.5 g/kg significantly decreased haemoglobin and platelet levels compared to the control ($P < 0.05$). All other parameters remained unchanged ($P > 0.05$) (Table 2). The biochemical analysis indicates that the polyherbal tea formulation at doses of 0.25 g/kg, 0.5 g/kg, and 1.0 g/kg significantly decreased levels of alkaline phosphatase, creatinine, urea, total cholesterol, and triglycerides in comparison to the control group ($P < 0.05$). Aspartate aminotransferase and albumin levels were significantly diminished at 0.5 g/kg relative to the control group ($P < 0.05$). Alanine aminotransferase levels were significantly diminished at doses of 0.25 g/kg and 0.5 g/kg in comparison to the control ($P < 0.05$). All other parameters remained unchanged ($P > 0.05$) (Table 3). Histological parameters demonstrate the impact of the polyherbal tea formulation on the heart, liver, kidneys, spleen, and lungs at dosages of 0.25 g/kg, 0.5 g/kg, and 1.0 g/kg, respectively. The polyherbal tea impacted the liver, leading to steatosis, but did not affect the heart, kidneys, spleen, or lungs in comparison to the control (Plates A to Z).

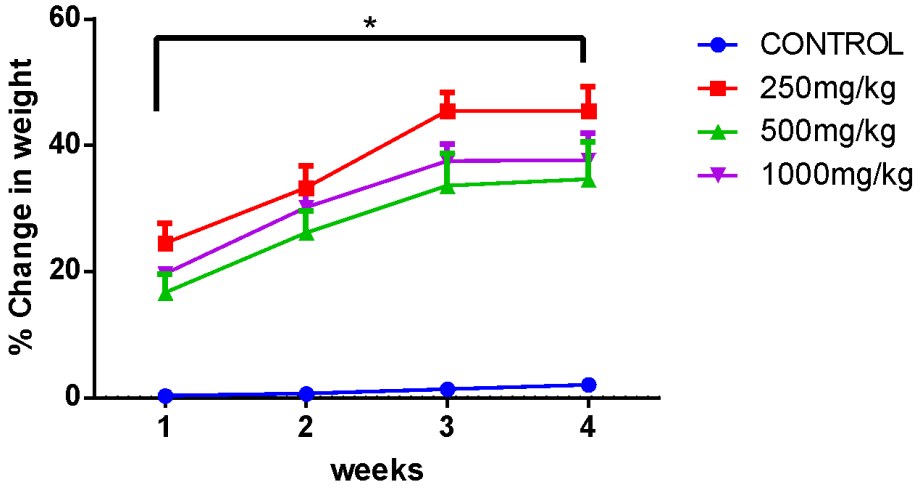


Figure 1: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza*garckeana, Anthocleista djalonensis, Allium sativum*

and Zingiber officinale) on percentage body weight change of the rats.

The polyherbal formulated tea at 250mg/kg, 500mg/kg and 1000mg/kg significantly increased the weights of the albino rats at weeks 1, 2, 3 and 4 respectively when compared with the control (*P < 0.05). Values are represented as Mean ± SEM, n = 5.

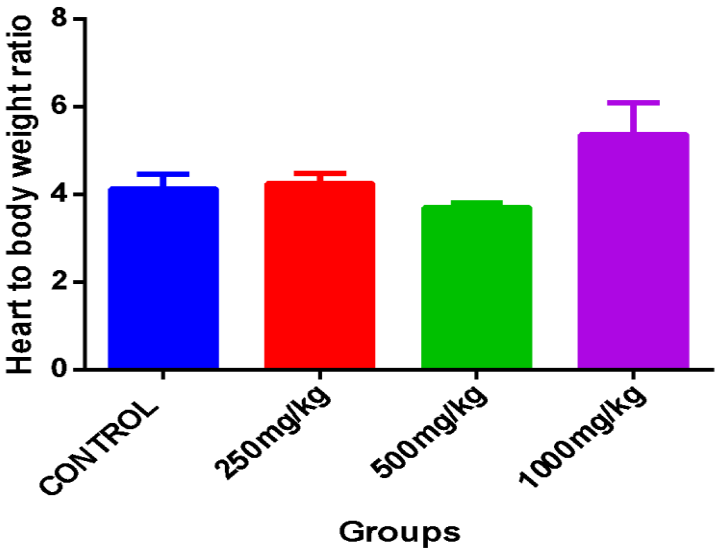


Figure 2: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum and Zingiber officinale)* on the heart to body weight ratio.

The poly-herbal formulated tea has no effect on the heart to body weight or on the heart when compared to control (P > 0.05). All values are represented as Mean ± Standard Error, n = 5.

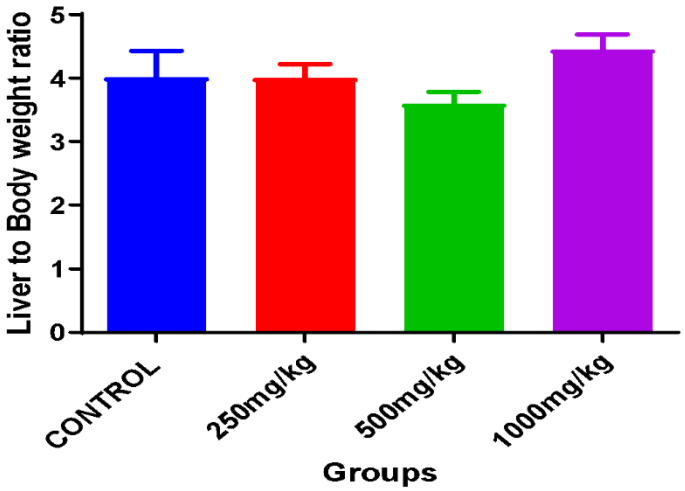


Figure 3: The effect of 28 days daily oral administration of the polyherbal

formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum and Zingiber officinale)* on the liver to body weight ratio.

The polyherbal formulated tea has no effect on the liver to body weight or on the liver when compared to control (P > 0.05). All values are represented as Mean ± Standard Error, n = 5.

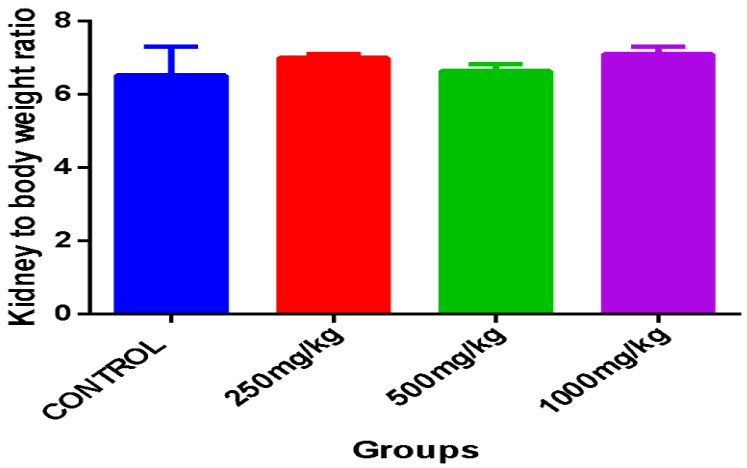


Figure 4: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum and Zingiber officinale)*on the kidney to body weight ratio.

The polyherbal formulated tea has no effect on the kidney to body weight or on the kidney when compared to control (P > 0.05). All values are represented as Mean ± Standard Error, n = 5.

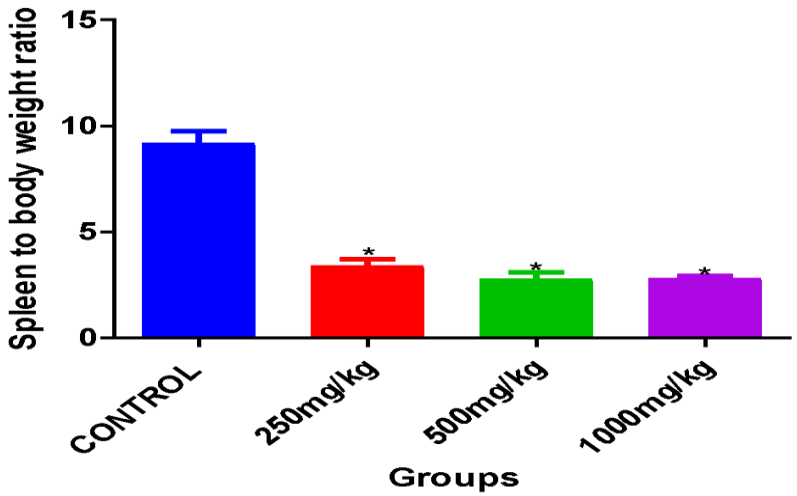


Figure 5: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum*

and Zingiber officinale) on the spleen to body weight ratio.

The polyherbal formulated tea significantly decreased the spleen to body weight or on the spleen at all dose levels when compared to control (*P <0.05). All values are represented as Mean ± Standard Error, n =

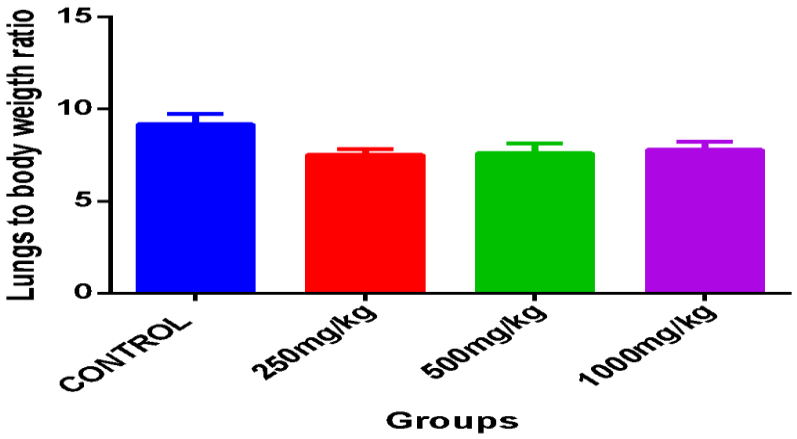


Figure 6: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum and Zingiber officinale)* on the lung to body weight ratio.

The polyherbal formulated tea has no effect on the lungs to body weight or on the lungs when compared to control (P > 0.05). All values are represented as Mean ± Standard Error, n = 5

Table 2: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum and Zingiber officinale)* on haematological parameters.

PARAMETER	CONTROL	0.25 g/kg	0.5 g/kg	1.0 g/kg
WBC (10^3/µl)	11.14 ± 5.531	4.60 ± 0.4604	5.340 ± 2.740	3.20 ± 0.1696
LYMP (%)	68.06 ± 6.578	84.03 ± 3.476	74.08 ± 3.528	76.84 ± 3.343
MID (%)	10.02 ± 1.125	6.440 ± 1.463	10.50 ± 1.967	9.030 ± 1.707
GRAN (%)	21.92 ± 5.671	9.530 ± 2.121	15.42 ± 2.083	13.69 ± 1.909
RBC (10^6/µl)	6.25 ± 0.2049	5.724 ± 0.3515	4.626 ± 0.669	6.457 ± 0.3142
HGB(g/dl)	10.88 ± 0.4893	9.890 ± 0.6408	7.860 ± 1.149*	10.70 ± 0.3889
HCT (%)	37.90 ± 1.422	36.75 ± 4.236	30.08 ± 4.236	40.84 ±1.604
PLT (10^3/µl)	441.4 ± 47.93	300.6 ± 49.51	175.0 ± 47.20**	303.4 ± 32.24

The polyherbal formulated tea at 0.5 g/kg significantly reduced hemoglobin level and platelet level when compared with control (*P < 0.05). All other parameters were not

affected (P > 0.05). Values are representing as Mean ± SEM, n = 5

WBC = White Blood Cells, LYMP = Lymphocytes, MID = Middle Cells and Percentage, GRAN = Granulocytes

RBC = Red Blood Cells, HGB = Haemoglobin, HCT = Haematocrit Value

Table 3: The effect of 28 days daily oral administration of the polyherbal formulated tea (*Ageratum conyzoides* , Azanza *garckeana, Anthocleista djalonensis, Allium sativum and Zingiber officinale)* on biochemical parameters.

PARAMETER	CONTROL	0.25 g/kg	0.5 g/kg	1.0 g/kg
ASP (U/L)	39.47 ± 9.626	23.42 ± 7.364	9.737 ± 2.175*	16.18 ±1.628
ALP (U/L)	67.92 ± 3.275	1.650 ± 0.7859**	12.10 ± 3.701**	18.01 ± 0.6875**
ALT (U/L)	14.34 ± 4.399	2.832 ±1.455*	1.758 ± 0.141*	4.812 ± 1.628
ALB (g/dL)	6.705 ±1.695	2.501 ± 0.9171	1.277 ± 2.5829*	2.395 ±0.4442
Cr (mg/dL)	11.85 ± 1.028	2.075 ± 0.4328**	2.350 ± 0.3227**	2.025 ± 0.75**
TP (g/dL)	17.34 ±1.225	9.881 ± 12.299	26.79 ± 12.11	4.804 ± 0.5753
UREA (mg/dL)	193.8 ± 8.452	34.73 ± 3.058**	67.85 ± 31.68**	34.73 ± 10.91**
URIC ACID(mg/dL)	34.50 ± 12.89	18.82 ± 3.324	17.45 ± 8.380	10.36 ±3.451
D. BILI (mg/dL)	4.368 ± 0.7008	4.580 ± 1.295	4.012 ± 0.6731	1.548 ± 0.2148
T. BILI (mg/dL)	19.35 ± 4.862	5.968 ± 1.883*	8.831 ± 2.057	0.6142 ± 11.90
CHOL (mg/dL)	404.8 ± 94.37	126.10 ± 11.28*	88.21 ± 20.60*	49.08 ± 3.685*
TRI (mg/dL)	582.0 ± 167.2	195.0 ± 36.19*	135.9 ± 52.14*	159.1 ± 28.96*
LDL (mg/dL)	94.98 ± 16.57	52.28 ± 30.55	33.15 ± 11.63	64.13 ± 26.57
HDL (mg/dL)	195.9 ± 40.11	118.2 ± 39.13	122.7 ± 53.12	348.3 ± 119.3

The polyherbal formulated tea at 0.25 g/kg, 0.5 g/kg and 1.0 g/kg significantly reduced Alkaline Phosphatase, Creatinine, Urea, Total Cholesterol and Triglyceride when compared to control (*P < 0.05). Aspartate aminotransferase and albumin were significantly reduced at 0.5 g/kg when compared to control (*P < 0.05). At 0.25 g/kg and 0.5 g/kg, Alanine aminitransferase was significantly reduced when compared to control (*P < 0.05). All other parameters were not affected (P >0.05). Values are represented as Mean ± SEM, n = 4

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, ALB = Albumin

TP = Total protein, D. BILI = Direct bilirubin, T. BILI = Total bilirubin , T. CHOL = Total cholesterol

TRI = Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein, Cr = Creatinine

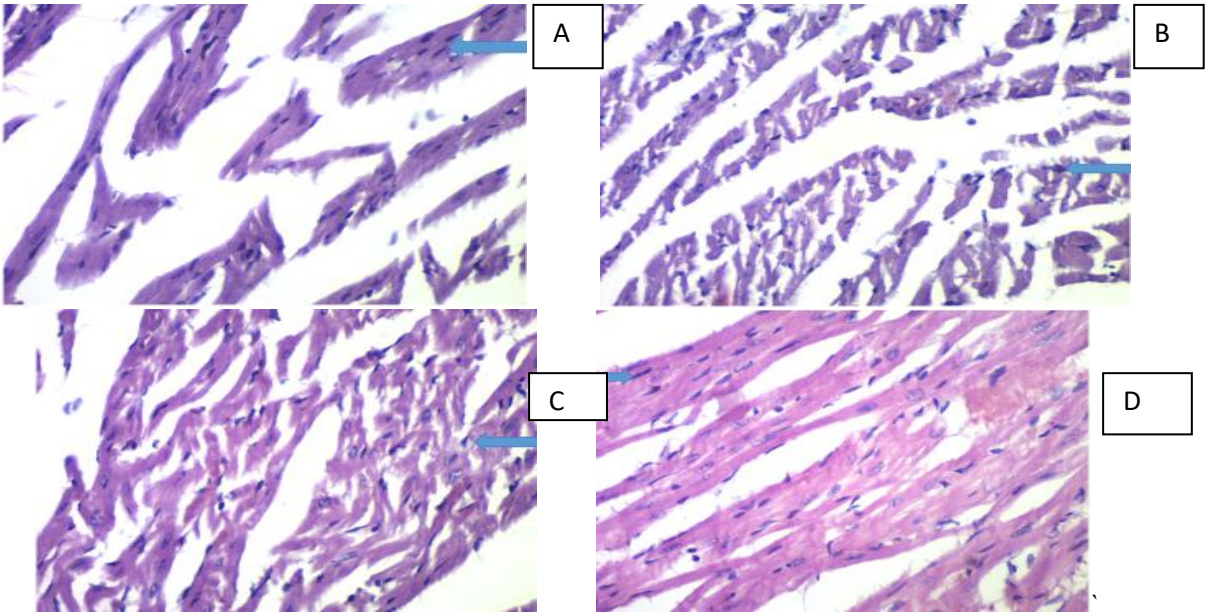
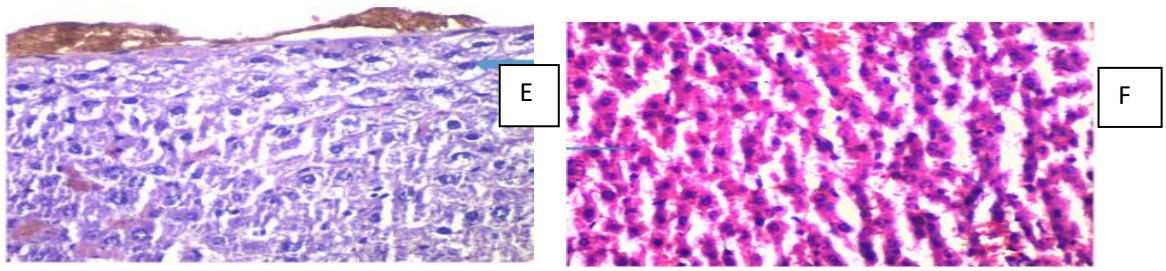


Plate A – Control: Section of cardiac muscle exhibiting myocytes with nuclei positioned peripherally, encircled by eosinophilic cytoplasm. Characteristics are consistent with normal myocytes.

Plate B – 0.25 grams per kilogram: A segment of cardiac muscle exhibits myocytes with nuclei located peripherally, encircled by eosinophilic cytoplasm. Characteristics are consistent with normal myocytes.

Plate C – 0.5 grams per kilogram: A segment of cardiac muscle exhibits myocytes with nuclei located peripherally, encircled by eosinophilic cytoplasm. Characteristics are consistent with normal myocytes.

Plate D – 1.0 g/kg: A region of cardiac muscle exhibits myocytes with nuclei positioned peripherally, encircled by eosinophilic cytoplasm. The characteristics align with typical myocytes.



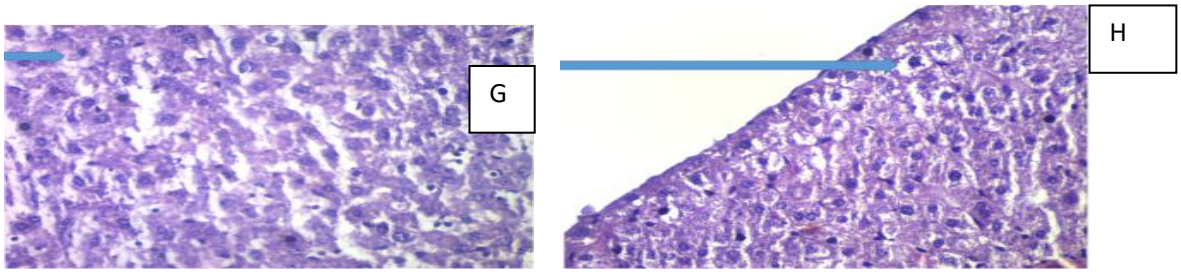


Plate E – Control: The liver examination shows hepatocytes (arrow) with eosinophilic cytoplasm surrounding centrally positioned normochromic nuclei that have indistinct nucleoli. characteristics typical of hepatocytes.

Plate F – 0.25 g/kg: Control: The liver section displays enlarged hepatocytes (marked by an arrow) characterised by cytoplasmic microvacuoles encircling centrally positioned normochromic nuclei with indistinct nucleoli. Characteristics correspond with the steatosis nucleus teatosis.

PlateG – 0.5 g/kg: Steatosis. Sectioning/kg: A liver specimen demonstrates hypertrophied hepatocytes (arrow), characterised by cytoplasmic microvacuoles surrounding a centrally positioned normochromic nucleus with indistinct nucleoli. The characteristics align with steatosis.

Plate H–1.0 g/kg: The liver section displays hypertrophied hepatocytes (arrow), characterised by cytoplasmic microvacuoles surrounding a centrally positioned, normochromic nucleus with indistinct nucleoli. The characteristics are indicative of steatosis.

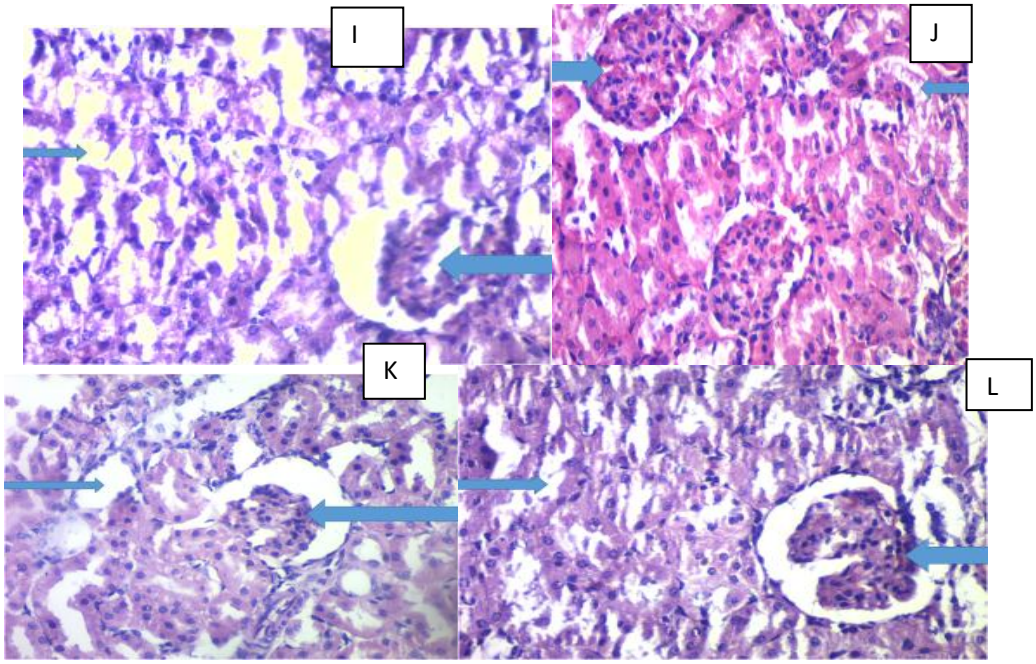


Plate I – CONTROL: The kidney section displays normal glomeruli (thick arrow) comprising typical mesangium, blood vessels, and epithelium. The tubules (thin arrow) are oval and lined with cuboidal epithelium, with certain tubules containing a pale eosinophilic substance. Characteristics are consistent with a normal kidney.

Plate J – 0.25 g/kg: A segment of the kidney exhibits normal glomeruli (thick arrow) comprising typical mesangium, blood vessels, and epithelium. The tubules (thin arrow) are oval and lined with cuboidal epithelium, with certain tubules containing a pale eosinophilic substance. Characteristics are consistent with a normal kidney.

Plate K–0.5 g/kg: A segment of the kidney exhibits normal glomeruli (thick arrow) comprising typical mesangium, blood vessels, and epithelium. The tubules (thin arrow) are oval and lined with cuboidal epithelium, with certain tubules containing a pale eosinophilic substance. Characteristics are consistent with a normal kidney.

Plate L – 1.0 g/kg: The kidney section exhibits normal glomeruli (thick arrow) comprising typical mesangium, blood vessels, and epithelium. The tubules (thin arrow) are oval and lined with cuboidal epithelium, with certain tubules containing a pale eosinophilic substance. Characteristics are consistent with a normal kidney.

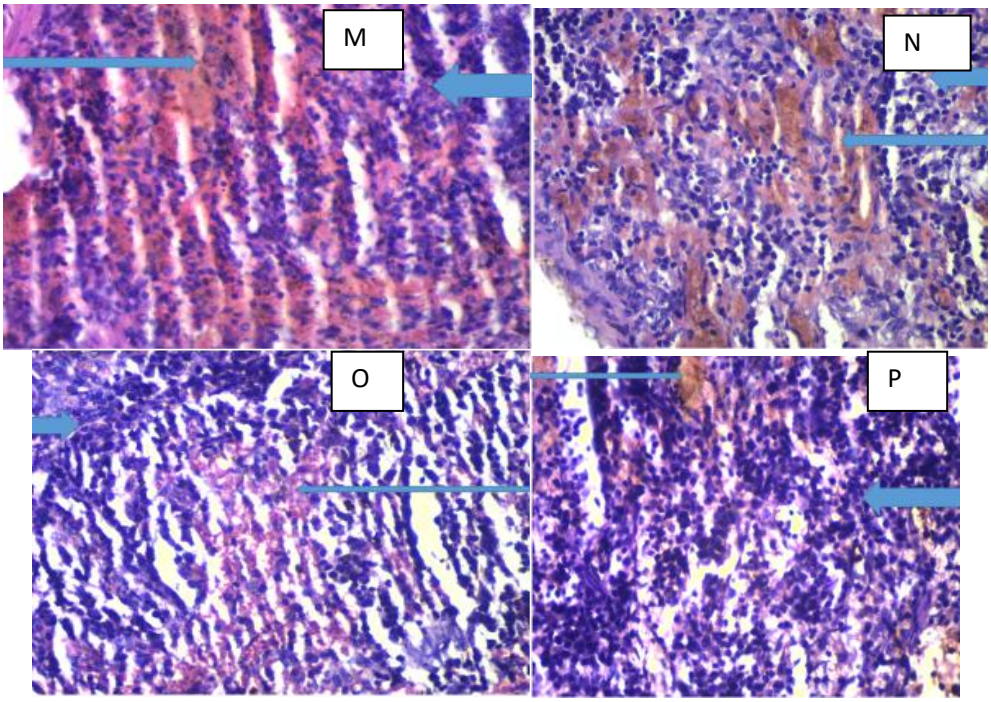


Plate M-Control: A segment of the spleen exhibits normal white pulp (thick arrow) predominantly composed of lymphocytes, alongside normal red pulp (thin arrow) rich in red blood cells. Characteristics are consistent with normal splenic tissue.

Plate N-0.25 g/kg: The spleen section exhibits normal white pulp (thick arrow) predominantly composed of lymphocytes and normal red pulp (thin arrow) containing numerous red blood cells. Characteristics are consistent with normal splenic tissue.

Plate O- 0.5 g/kg: A portion of the spleen exhibits normal white pulp (thick arrow) primarily composed of lymphocytes and normal red pulp (thin arrow) containing numerous red blood cells. Characteristics are consistent with normal splenic tissue.

Plate P- 1.0 g/kg: A portion of the spleen exhibits normal white pulp (thick arrow) primarily composed of lymphocytes and normal red pulp (thin arrow) containing numerous red blood cells. Characteristics are consistent with normal splenic tissue.

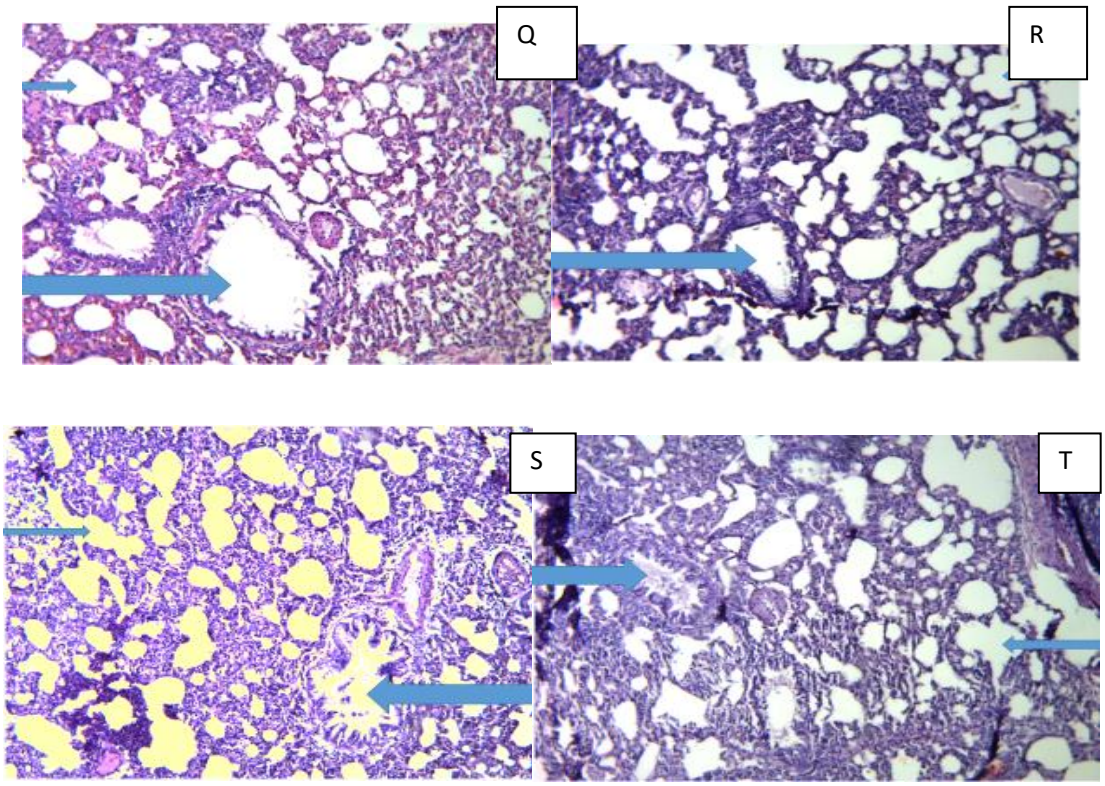


Plate Q - Control: Sections of the lung show a normal airway (thick arrow) and alveoli sacs (thin arrow) that are separated by the interstitium. Features are in keeping with **normal lungs.**

Plate R - 0.25 g/kg: Sections of the lung show a normal airway (thick arrow) and alveoli sacs (thin arrow) that are separated by the interstitium. Features are in keeping with **normal lungs.**

Plate S 0.5 g/kg: Sections of the lung show a normal airway (thick arrow) and alveoli sacs (thin arrow) that are separated by the interstitium. Features are in keeping with **normal lungs.**

Plate T – 1.0 g/kg: Sections of the lung show a normal airway (thick arrow) and alveoli sacs (thin arrow) that are separated by the interstitium. Features are in keeping with **normal lungs.**

DISCUSSION

An acute toxicity study determines the LD50, the dose of a test substance that results in mortality in 50% of the test population during short-term exposure, to evaluate the substance's safety (Ozolua *et al.*, 2010). The LD50 of the polyherbal formulated tea was indeterminate in this study, as no mortality occurred in the mice during the acute toxicity test, even at the maximum dosage of 10 g/kg. As per the safety scale referenced by Ozolua *et al.* (2009), a substance is deemed non-toxic if the LD50 exceeds 5 g/kg. This indicates that the aqueous extract of the tea possesses a comparatively broad safety margin (Angeles-López *et al.*, 2010; Nworgu *et al.*, 2009). Signs of toxicity from the polyherbal formulated tea extract include regular paw licking, writhing reflex, stretching, drowsiness, tachypnoea, restlessness, and jerking at dosages of 1.0 g/kg, 5 g/kg, 7.5 g/kg, and 10 g/kg, respectively (Table 1).

A subacute toxicity test is performed to assess a new drug's potential negative effects after prolonged administration. The impact of a substance on animal body weight is less significant with brief exposure compared to prolonged exposure (Wang *et al.*, 2019). This study demonstrated that the polyherbal tea formulation, at all dosage levels, enhanced the body weight of the animals during the entire administration period (Figure 1). Body weight variation serves as a toxicity assessment metric in toxicological research (Vahalia *et al.*, 2011). This effect may be ascribed to the capacity of the formulated polyherbal tea to enhance appetite, metabolism, and antioxidant levels, as documented by Oluba et al. (2010). Determining the organ-to-body-weight ratio of the experimental animals is essential to evaluate the treatment's impact on the various organs (Michael *et al.*, 2007). This study demonstrated that the poly-herbal tea formulation reduced the spleen-to-bodyweight ratio in rats across all dosage levels (Figure 5), while exerting no influence on the organ-to-bodyweight ratios of the heart, liver, kidneys, and lungs (Figures 2, 3, 4, and 6). Nirogi et al. (2014) suggest that variations in organ-to-body-weight ratios may result from inter-animal variability, stress, and physiological factors. The polyherbal formulation is safe for internal organs.

Haematological parameters are used in clinical practice to assess health and disease conditions; they are important tools for diagnosing health disorders, drug toxicity or side effects and are biomarkers to assess disease progression or response to therapy (Koronowicz *et al.,* 2016; Miri-Dashe *et al.,* 2014; Adetifa *et al.,* 2009). In this research, the

polyherbal formulated tea reduced the platelet count and haemoglobin level at 0.5 g/kg; all other parameters were not affected **(Table 2)**. A low haemoglobin level may indicate an anaemic condition; according to Gotoh *et al.* (2015) and Kishimoto *et al.* (2020), a significant increase in the haemoglobin and haematocrit values are risk factors for cardiovascular disease development, but since there was a significant decrease in the haemoglobin and no effect on the haematocrit value, the extract may be said to have a cardioprotective effect. Platelets maintain blood haemostasis (Gotoh *et al.,* 2015; Radaelli *et al.,* 2007). The decreased platelet count may indicate that the polyherbal formulated tea has anti-platelet properties and, hence, may be effective in the treatment of thrombocytosis. However, further studies should be done to elucidate this finding. Since no effect was observed in the other haematological parameters, it indicates that the extract did not induce any immuno-stimulatory response in the rats, as these cells are usually elevated in response to foreign agents and are known to produce antibodies that allow the destruction of infectious cells (Juliet *et al.,* 2023).

Testing biochemical parameters is essential, as it yields vital information regarding the organs and tissues within the body, along with the metabolic condition of the animal. Alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels are routinely employed to assess the degree of cellular injury and function as significant biomarkers of hepatic function. (Uwaya and Idu, 2022). The increased levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase signify hepatic damage (Adeyemi *et al.*, 2015; Liu *et al.*, 2014; Gowda *et al.*, 2010). This study demonstrated that the polyherbal tea formulation reduced alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels, suggesting potential hepatoprotective properties of the aqueous extract. A notable reduction in total bilirubin and albumin was observed at a dosage of 0.5 g/kg of the formulated tea. The polyherbal tea formulation reduced levels of urea, uric acid, and creatinine, which may indicate improved kidney function; thus, the extract may be considered nephroprotective or non-toxic to the kidneys (Rawal *et al.*, 2020; Gowda *et al.*, 2010). The polyherbal tea reduced total cholesterol and triglyceride levels, suggesting that the aqueous extract may regulate lipid metabolism and have a favourable lipid profile. A high lipid profile increases the risk of atherosclerotic cardiovascular disease (ASCVD), which can lead to heart attack or stroke, implying potential cardioprotective properties (Juliet 2023; Olayinka 2021; Meral *et al.*, 2015). All other biochemical parameters remained unchanged.

Histopathology in toxicity studies is essential for assessing the impact of treatment on different organs; therefore, it is included among the parameters evaluated in the toxicological studies of a substance (Histol J. 2014). This study's histopathological examination of various organs, Plates A to T, revealed normal organ characteristics, except for Plates F, G, and H, which exhibited steatosis. Consequently, the aqueous extract did not impact the organs; however, extreme caution is advised when the extract is consumed over an extended duration (Uwaya and Idu, 2022).

CONCLUSION

This study demonstrates that the aqueous extract of the polyherbal tea formulation is safe. The tea may exhibit hepatoprotective, nephroprotective, and cardioprotective properties. Additional research is necessary to clarify these findings.

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Conflict of interest

The authors assert the absence of any conflict of interest.

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