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Toxicological implications of the fruit of Harungana madagascariensis on wistar rats

Olusayo Aderonke Shorinwa* and Barizonmdu Monsi

Abstract

Background: The unopened buds of the fruit of *Harungana madagascariensis* is used in the treatment of anaemia and skin diseases in traditional medicine. Hence, this study aims to scientifically evaluate the effects of oral administration of the fruit extract of *Harungana madagascariensis* on haematological, biochemical and histological parameters in Wistar rats.

Methods: Phytochemical screening of the ethanol fruit extract of *H. madagascariensis* was carried out. Acute toxicity test was done using Lorke's method. Sub-acute toxicity studies were done using 24 rats of both sexes which were randomized into four groups of six rats each. Animals in groups A, B, C were administered with the extract at doses of 250, 500 and 1000 mg/kg, respectively while group D animals were given distilled water (5 mg/kg) and served as the control group. All administrations were done through the oral route for 30 consecutive days. Body weights of the animals were taken weekly during the study. The animals were sacrificed under diethyl ether anaesthesia and blood samples collected for evaluation of haematological (red blood cell, haemoglobin, packed cell volume and white blood cell) and biochemical (alanine transferase, alanine aminotransferase, alkaline phosphatase, urea, creatinine, total cholesterol and total protein) parameters. Histological examination was conducted on the liver and kidney of the animals.

Results: Preliminary phytochemical screening of the extract revealed the presence of alkaloids, anthraquinones, steroidal nucleus, saponins, carbohydrates, flavonoids, and tannins. Acute toxicity test showed that the LD_{50} was greater than 5000 mg/kg. There was no statistically significant ($P < 0.05$) difference in the RBC, HB, PCV and WBC of the extract treated groups when compared to the control group. There was however, a statistically significant ($P < 0.05$) difference in the creatinine level of the 500 mg/kg extract –treated group and the control. There was no statistically significant ($P < 0.05$) difference in other biochemical parameters of the extract treated groups and the control group except for a marginal increase in the total protein in the group treated with 1000 mg/kg of the extract (60 g/L) compared with control (54.80 g/L). Histopathological examination showed alterations in the morphology of the liver and kidney in extract treated groups as compared to the control groups.

Conclusion: The findings have revealed that the ethanol fruit extract of *H. madagascariensis* should be used with caution especially during prolonged usage as the histology showed it has nephrotoxic and hepatotoxic potentials. Further studies will be done to establish the effects of the extract on white blood cells.

Keywords: *Harungana madagascariensis*, Ethanol, Haematological, Biochemical, Histology

* Correspondence: olusayo.shorinwa@uniport.edu.ng; sayoshorinwa@yahoo.com
Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

Background

Medicinal plants consist of various types of plants used in herbal medicine and are considered as a rich source of ingredients which can be utilized in the development and synthesis of new drugs e.g. ginger which is used as an antioxidant supplement [1]. Medicinal plants are indeed a primary source of health care for 80% of the world's population in the form of plant extracts or their active components as a result of their properties [2].

Medicinal plants have a promising future because there are about half a million plants around the world, most of whose medicinal activities have not been investigated, and whose medicinal activities could be utilized in the treatment of present diseases [3]. Herbal medicines are regarded by some people, especially in the developing countries to be less damaging to the human body than orthodox drugs and therefore may be considered as safe [4]. However, this is not always true, as some herbal products have been reported to have toxic effects. Reports of toxicological studies on Saffron (*Crocus sativus* L.), a medicinal plant and food spice with its constituents reviewed by [5] suggested that pregnant women should avoid using high dose of saffron because it has been found that exposure to high levels of this plant may increase miscarriage rate in pregnant females, even though it selectively inhibited cancer cell proliferation while it didn't exert toxic effect on normal cells. This further strengthens the need for scientific evaluation of medicinal plants in use.

The public's increasing demand for alternative medicine, the newly found global interest in phytomedicine and herbal therapies, the rising cost of conventional prescription drugs, have led to rapid rise in the use of unregulated herbal supplements and therapies, largely outside the United States [6]. Given the large number of herbal products on the market and the relatively low budget available for research to date, safety assessment in accordance with modern guidelines has been carried out on relatively few herbs [7].

Toxicity assessment helps in decisions as to whether a new drug should be approved for clinical use or not [8]. The earliest report of the toxicity of herbs originated from Galen, a Greek pharmacist and physician who showed that herbs contain not only medicinally beneficial constituents, but may also consist of some harmful substances [9]. *Harungana madagascriensis* is native to Madagascar, Mauritus and tropical Africa where it grows on the margin of wet forests and regrows after disturbance [10]. Different parts (stem bark, roots, leaves and fruits) of *H. madagascariensis* are used in traditional medicine for various purposes.

H. madagascariensis' unopened buds are traditionally used in the treatment of skin disease and anaemia. Shorinwa et al. [11] reported that the ethanol extract of the fruit of *Harungana madagascariensis* possesses anti-anaemic activity. *Harungana madagascariensis* is a component of Jubi Formula, a herbal preparation which was found to restore the packed cell volume (PCV), and haemoglobin (Hb) concentration in anaemia conditions and is a potential substitute for blood transfusion [12]. However, several scientific studies had reported that the ethanolic extract of *H. madagascariensis* possesses centrally- and peripherally-mediated analgesic properties [13], antioxidant and anti-modulatory effects [14] and hypotensive and cardioprotective effects [15, 16].

A recent study conducted by Ndjakou et al. [17], showed the anti-plasmodial effects of six isolated compounds from the root bark of *H. madagascariensis* in vitro, which also justifies the use of the plant in traditional medicine for the treatment of malaria.

However, considering the numerous potential beneficial effects of *H. madagascariensis*, no toxicological studies concerning the safety profile of the extract of the fruit of this plant have been reported. Hence, this research was carried out to evaluate the effects of the ethanol exact of the fruit of *H. madagascariensis* on heamatological, biochemical and histological indices of Wistar rats.

Methods

Plant materials

The fruit of *H. madagascariensis* was collected in September, 2015 from the medicinal plant garden of the Faculty of Pharmaceutical Sciences, in the University of Port Harcourt, Rivers State, Nigeria. Identification and authentication of the plant were done by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology and a specimen voucher with herbarium number (UPH/P/080; UPH/V/1219) was deposited at the herbarium for reference.

Extraction of plant materials

The fruits of *Harungana madagascariensis* were cleaned, air dried under shade, not directly exposed to sunlight for about 3 weeks and ground into fine powder using a grinding machine and kept in an airtight plastic container. One hundred and forty grams of the powder was macerated using 4 L of absolute ethanol for 72 h. The menstruum was filtered through Whatmann No. 1 (Whatmann International Limited, Maidstone, UK) filter paper and concentrated to dryness over a water bath at 40 °C. The extract obtained was weighed, the percentage yield determined and stored in an airtight container, refrigerated and used for the study.

Chemicals and reagents

Absolute JHD ethanol manufactured by Guangdong Guanghua Science Technology Limited China was used exclusively for the extraction.

Preliminary qualitative phytochemical screening

The ethanol extract of the fruit of *H. madagascariensis lam* was screened for the presence of plant constituents as described for alkaloids [18], saponins and phlobatannins [19], flavonoids, tannins, anthraquinones,, carbohydrates and triterpenes/ steroids [20] as follows:

Alkaloids

Plant extract (0.5 g) was stirred with 5 ml of 1% v/v aqueous hydrochloric acid on water bath and then filtered. Distilled water was added to the residue and 1.0 mL of the filtrate was treated with two drops of Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (solution of iodine in potassium iodide), and Dragendorff's reagent (solution of potassium bismuth iodide). The formation of a cream colour with Mayer's reagent, reddish-brown precipitate with Wagner's and Dragendorff's reagents give a positive test for alkaloids.

Saponins

About 1 g of the extract was boiled in 5 ml of distilled water and filtered. The filtrate was shaken vigorously for 5 min for a stable persistent froth which confirms the presence of saponins.

Phlobatannins

The plant extract was stirred with water and filtered. The filtrate was boiled with 10% v/v hydrochloric acid. Deposition of a red precipitate was taken as a positive test for phlobatannins.

Flavonoids

A little quantity of the plant extract was dissolved with concentrated hydrochloric acid and then filtered. To the filtrate was added few pieces of magnesium ribbon. The formation of orange, crimson, or magenta colouration indicated the presence of flavonoids.

Tannins

Plant extract (0.5 g) was boiled with distilled water and filtered, then 5% w/v ferric chloride reagent was added. A blue black precipitate was taken as an evidence for the presence of tannins.

Free anthraquinone

The plant extract (0.5 g) was shaken with 10 ml chloroform and filtered. 10% v/v ammonia solution was added to the filtrate and the mixture shaken. The presence of pink, red, or violet colour in the ammoniacal layer (lower phase) indicated the presence of free anthraquinones.

Combined anthraquinones

The plant extract (0.5 g) was boiled with 10 ml of aqueous sulphuric acid and filtered hot. The hot filtrate was shaken with 5 ml chloroform. The top chloroform layer was reduced to half its volume. 10% v/v ammonia solution was added. The presence of pink, red, or violet colour in the ammoniacal layer (lower phase) indicated the presence of combined anthraquinone or anthraquinone derivative.

Carbohydrate

The plant extract (0.5 g) was dissolved in water and to a 2 ml portion were added 2 drops of 10% napthol and 2 ml of concentrated sulphuric acid solution. A violet colour observed indicated the presence of carbohydrate.

Triterpenoids/steroids

Plant extract (0.5 g) was mixed with 1 ml of acetic acid anhydride and dipped in ice, and then 1 ml of concentrated sulphuric acid was carefully poured down the wall of the test tube to form a layer. A colour change from violet to blue to green indicated the presence of steroidal nucleus (aglycone portion of cardiac glycoside), a pink – red colour indicated the presence of a triterpenoid nucleus.

Experimental animals

Adult Wistar rats (weighing130–200 g) of either sex maintained at the animal house of the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, were used for the study. The animals were fed ad libitum with standard feed (Broiler finisher- Guinea feeds) and had free access to water. The animals were acclimatized for 2 weeks before the commencement of the study. Ethics approval was obtained from the Research ethics committee of the University of Port Harcourt.

Acute toxicity evaluation

The method of Lorke [21] was used for the evaluation of acute toxicity. The 18 animals selected for the acute toxicity studies were divided into six groups of three animals each. The animals were fasted overnight before commencement of administration of the extract. The first three groups were treated with 10, 100, and 1000 mg/kg body weight of the ethanol extract of the fruit of *H. madagascariensis* lam, respectively. The animals were kept under observation for 24 h for signs of toxicity and death. After 24 h, none of the animals died or adverse effect was recorded. Then the remaining three groups of the animals were given doses of the extract at 1600, 2900, and 5000 mg/kg, respectively and observed for further 24 h [21].

Sub-acute toxicity study

Twenty four rats of both sexes were selected randomly and then divided into four groups of six rats each.

Animals in groups A, B, C were administered with the extract at doses of 250, 500 and 1000 mg/kg, respectively (which corresponds to 1/20th, 1/10th, and 1/5th of the 5000 mg/kg dose) while group D animals were given distilled water (5 mg/kg) and served as the control group. All drug administration was done consecutively for 30 days after which the animals were then anaesthetized with diethyl ether and sacrificed.

Weekly body weight

The body weight of each rat used was assessed using a sensitive balance (Ohaus CS200 Compact scale, Germany) before commencement of dosing, every seventh day during the dosing period and on the day of sacrifice which corresponded with the 0, 7th, 14th, 28th, and 30th day, respectively.

Hematological assay

After 30 days of administration of the fruit extract, blood samples were collected into a heparinized tube and were subjected to hematological analysis. The following hematological parameters were determined; red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HB), packed cell volume (PCV) [22].

Biochemical analysis

Serum urea was determined using the Diacetylmonoxime method [23]. Creatinine was determined with QCA kit using Jaffe's method, while Alanine amino transferase (SGPT) and Aspartate amino transferase (SGOT) were determined using Randox laboratory Kit (England) method which is a colorimetric method [24, 25]. Total cholesterol and alkaline phosphatase were measured using the enzymatic end point method [26–30], while the total protein was determined using the burette method [23].

Relative organ weight

On the 30th day (D_{30}) of the dosing period, all the animals were euthanized by exsanguinations under diethyl ether-induced anesthesia. The liver and kidneys were carefully extracted out and weighed in grams (absolute organ weight) as described by Uma et al. [31]. The relative organ weight of the organs was then calculated using the equation:

$$\text{Relative Organ Weight} = \frac{\text{Absolute Organ Weight (g)}}{\text{Body weight of rat on sacrifice}} \text{ X } 100$$

Histopathology

Histopathological investigation of the liver and kidney, were done according to the method of Pieme et al. [32]. The organ pieces of about (3–5 μm thick) were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50 °C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. Appropriate microscopic examination was carried out in the organs of both the control and treated groups.

Statistical analysis

Data obtained from the experiments are expressed as mean ± standard error of mean (SEM). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) and Student's t-test was done to determine the significance of difference between the control groups and the treated groups. Graph Pad Prism version 5.0 (San Diego, California, USA) was used. *P*-values< 0.05 were considered to be statistically significant.

Results

Relative organ weight

There were no statistically significant changes in the relative weights of the organs, both the liver and the kidney between the control group and the extract treated group (Table 6).

Histopathology of the liver

The liver tissue of the control group showed normal histological architecture (Fig. 1a).

The liver tissue of the 250 mg/kg treated group showed mild inflammatory changes, while inflammation of the periportal tract was revealed in the 500 mg/kg group and marked periportal inflammation was observed in the group treated with 1000 mg/kg of the plant extract after 30 days (Fig. 1b, c and d).

Table 1 Preliminary phytochemical screening of ethanol extract of fruits of *H. madagascariensis* lam

Chemical constituent	Observation
Anthraquinones	+
Aglycone	+
Steroids	+
Saponins	+
Carbohydrates	+
Flavonoids	+
Tannin	+
Phlobatanin	–
Combined anthraquinones	–
Alkaloid	+

+: Present; – Absent

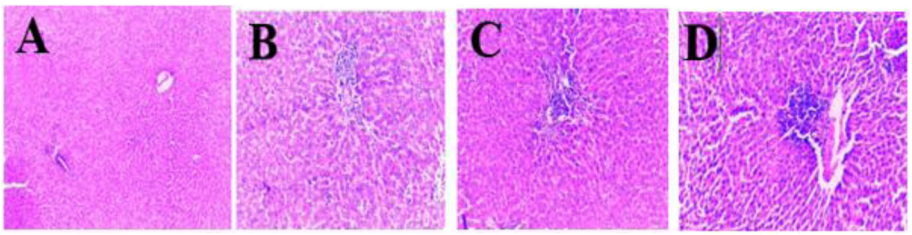

Fig. 1 a-d Shows photomicrographs of liver tissue of rats. **a** (control): A cross section of the liver in control rat with the arrows showing the hepatocyte sinusoid and the portal tract having normal histological appearance (×100 magnification). All sections were stained with haematoxylin and eosin. **b** A cross section of the liver of rats treated with 250 mg/kg of the ethanol extract of *H. madagascariensis lam*. Showing mild inflammatory changes (×200 magnification). All section stained with hematoxylin and eosin. **c** A cross section of the liver in rat treated with 500 mg/kg of ethanol extract of *H. mdagascariensis* with arrow showing inflammations of the periportal tract (×200 magnification). All section stained with haematoxylin and eosin. **d** A cross section of liver in rat treated with 1000 mg/kg of ethanol extract of *H. madagascariensis* lam. With arrow showing marked periportal inflammation (× 200 magnification). All section stained with haematoxylin and eosin

Histopathology of the kidney

There was no change in the histological features of the kidney of the rats after 28 days of administration of the acetone extract of *Crinum jagus* bulbs (Fig. 2a).

The kidney tissue showed congestive changes at 250 mg/kg while dilated and congestive vessels were noticed at 500 mg/kg and 1000 mg/kg respectively (Fig. 2b, c and d).

Discussion

Traditionally, the leaves and stem bark of *Harungana madagascariensis* are used for the treatment of anaemia, the stem bark is also used for nephrosis, malaria, gastro-intestinal disorders and fever [33–35]. Traditionally, herbal products are considered non-toxic and have been used by the general public and traditional healers world-wide to treat various ailments. However, the fact that a herbal preparation is of natural origin does not necessarily make it safe. The active ingredients of plant extracts are chemicals like those of synthetic or purified drugs. In low amounts, they may be ineffective, while in the right amounts, they may prove beneficial. When large quantities are used for a prolonged period, plant extracts may be injurious to health and cause serious toxic effects [36]. The phytochemical screening of the plant extract revealed the presence of anthraquinone, aglycone, alkaloids, steroidal nucleus, saponins, carbohydrate, flavonoids, tannin, while phlobatannin was absent. This can be related to a study conducted by Omotayo and Temitope [37] which revealed the presence of all the above mentioned compounds in the ethanol extract of *Harungana madagascariensis*.

Ethanol was used for extraction so as to avoid any form of contamination, enhance the concentration of the extract to dryness and to ensure the stability of the extract throughout the study period even though it was stored in a refrigerator. Ethanol has also been known to be safe for human consumption (food and drug journal). Previous scientific studies on stem bark of this plant used ethanol solvent for extraction [13, 14].

During the 30 days of administration and observation, there was a statistically significant ($P < 0.05$) increase in the body weight of the rats used in the study. Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. It can also be used to explain blood related effects of chemical compounds or plant extracts [38]. The result of the hematological assay showed that there was a slight increase in the packed cell volume, hemoglobin concentration and number of red blood cells between the extract treated group as compared to the control group. This was however, not dose-dependent, with the group receiving 1000 mg/kg producing a marginal increase which was not statistically significant ($P > 0.05$). There was no significant change in the white blood cell count. The slight increase in these

Table 2 Acute toxicity studies. There was no death recorded in all the groups during the two phases according to Lorke's method

Group	No. of Rats Used	Dose of extract(mg/kg)	Number of dead Rats	Percentage (%) cumulative dead rats
1	3	10	0	0
2	3	100	0	0
3	3	1000	0	0
4	3	1600	0	0
5	3	2900	0	0
6	3	5000	0	0

Table 3 Effects of ethanol fruit extract of *H. madagascariensis* on body weight (Mean ± S.E.M). During the 30 days of study and observation, there was a statistically significant ($P < 0.05$) change in the body weight of the rats in all the group

Treatment	Dose (mg/kg)	Body weight		% increase in body weight
		Day 0	Day 30	
Extract	250	110.93 ± 6.22	179.48 ± 9.12[a]	61.8
Extract	500	115.07 ± 0.89	165.82 ± 6.54[a]	44.1
Extract	1000	127.22 ± 5.10	168.76 ± 13.69[a]	32.65
Control	5 ml/kg	108.62 ± 1.67	154.9 ± 7.34[a]	42.6

parameters may be due to stimulatory effect of the extract on the production of hematopoietic regulatory elements such as erythropoietin and colony – stimulating factors by the stromal cells and macrophages in the bone marrow. A decrease in white blood cell numbers observed in this study might be due to increased tissue demand as can be seen in severe inflammatory responses, endotoxemia, or bone marrow suppression [39].

Further studies will be carried out to look into the decrease in the white blood cell numbers observed in this study. The result of the biochemical analysis showed that there was no statistically significant ($P > 0.05$) increase in the level of AST, ALT and ALP in any of the extract treated groups as compared to the control group. Biapa et al. [40] also conducted a study on the hydroethanol extract of the stem bark of *Harungana madagascariensis* on rats and mice, and stated that *H. madagascariensis* caused a dose-dependent increase in alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST). He also stated that the above named parameters were only found to increase significantly $P < 0.05$ at higher doses of about 1.25 and 2.5 g/kg, which is far higher than the highest dose of 1000 mg/kg used in the study. This also implies that a higher dose of the fruit extract of *H. madagascariensis* may likely produce a statistically significant increase in the ALT, ALP and AST.

Serum AST and ALT are useful indices for identifying inflammation and necrosis of the liver [41]. ALT is a cytoplasmic enzyme that is found in high concentration in the liver, an increase in the serum levels of this specific enzyme indicates hepatocellular damage. Although AST is less specific than ALT as a marker of liver damage, elevation in the serum levels of these two enzymes is an indicator of tissue damage and altered membrane permeability [40, 42]. ALT has higher concentration in the liver, with kidney and skeletal muscles having lesser activity of the enzyme. ALT values are usually greater than AST activity at early or acute hepatocellular disease [43]. Alkaline phosphatase (ALP) is a marker of obstructive jaundice and intrahepatic cholestasis [44].

There was no significant alteration in the level of blood urea of the extract treated rats as compared to the control group. There was however, a statistically significant ($P < 0.01$) increase in the creatinine level of the group administered with 500 mg/kg of the extract as compared to the control. A rise in serum creatinine level indicates that less creatinine is filtered by the kidney. This means that the kidneys may not be functioning efficiently as they ought to. The total protein measures the total amounts of albumin and globulin present in the blood. There was also a slight increase in the total protein in the group treated with 1000 mg/kg of the extract,

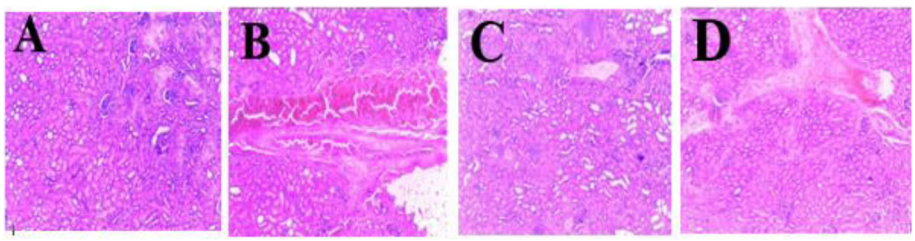

Fig. 2 a-d Shows photomicrographs of kidney tissue of rats. **a** (control): A cross section of kidney in control rat with arrow showing normal histological appearance of the glomerulus and the tubules (× 100 magnification). All sections were stained with haematoxylin and eosin. **b** A cross section of the kidney of rat treated with 250 mg/kg of the ethanol extract of *Harungana madagascariensis* with arrow showing congestive changes (× 200 magnification). All sections were stained with haematoxylin and eosin. **c** A cross section of the kidney of rat treated with 500 mg/kg ethanol extract of *H. madagascariensis* lam with arrow showing dilated and congested vessel. (×200 magnification). All sections were stained with haematoxylin and eosin. **d** A cross section of the kidney in rat treated with 1000 mg/kg ethanol extract of *H. madagascariensis lam* with arrow showing dilated and congested vessels. All sections were stained with haematoxylin and eosin. (×200 magnification)

Table 4 Effects of ethanol fruit extract of *H. madagascariensis* on hematological parameters in wistar rats. (Mean ± S.E.M). There was no statistically significant ($P > 0.05$) difference in the red blood cell count, packed cell volume, hemoglobin concentration and white blood cell count values of the extract treated groups when compared to the control. However, there was a marginal decrease in the white blood cell count of the treated groups compared to the control

Treatment	Dose (mg/kg)	Parameters			
		RBC($\times 10^{12}$/L)	PCV (%)	HB (g/dL)	WBC ($\times 10^{9}$/L)
Extract	250	4.64 ± 0.30	41.00 ± 2.47	13.70 ± 0.82	8.98 ± 1.10
Extract	500	4.20 ± 0.31	37.40 ± 3.16	12.52 ± 1.04	8.10 ± 1.30
Extract	1000	5.02 ± 0.31	44.80 ± 2.52	14.94 ± 0.84	7.76 ± 0.80
Control	5 ml/kg	4.22 ± 0.21	38.00 ± 2.00	12.76 ± 0.67	10.90 ± 2.41

$P > 0.05$. Values were not statistically significant ($P > 0.05$) as compared to control. $n = 5$, t-test $P < 0.05$

however this was not statistically significant ($P > 0.05$) when compared to the control group.

There were no statistically significant change in the relative weight of the liver and the kidney between the control and extract treated groups. In sub-acute toxicity studies, the internal organ weight changes serve as an indicator of adverse effects and also an important index of physiological and pathological status of animals [45]. The relative organ weight helps to indicate that the organ was exposed to injury or not [46].

The result of histopathological examination revealed the presence of inflammatory cells in the portal tract of all the extract treated rats. While the degree of inflammation increased with increased dose of the extract with the group receiving 1000 mg/kg showing marked periportal inflammation. Also the histology of the kidney showed a normal histological appearance in the control groups while there was presence of dilated and congested vessels in all the extract treated groups, this was however found to be dose dependent. The histopathological changes produced by the fruit extract may be due to the presence of saponin in the plant extract. This also correlates with the findings of Cherian et al. [47], who had reported the toxic action of saponin in rats.

This study also relate to some extent the research conducted by Biapa et al. [40], on the hydroethanolic extract of the stem bark of the plant on the histology of the liver of rats who reported a dose dependent alteration in the histology of the liver. He reported the inflammation, degenerated hepatocytes, and congestion at higher doses of (1.25 and 2.5 g/kg). This was however different from the study conducted as there were inflammations of the portal tract and congestion even at a lower dose of about 250 mg/kg administered. Although the later study was performed on the fruit of the plant which is different from the stem bark used in the previous study. Therefore there may be possibilities of varying degree of toxicity of different parts of a particular plant. This means that some plants may contain more toxic constituents in certain parts of the plant than others.

Although *Harungana madagascariensis* has been found to have numerous health benefits, care has to be taken upon its prolonged use in the treatment of various disease conditions considering its potential toxic effect on the histology of liver and kidney.

Table 5 Effects of ethanol fruit extract of *H. madagascariensis* on biochemical parameters in Wistar rats (Mean ± S.E.M). The extract produced no statistically significant ($P > 0.05$) difference in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) in the extract treated groups when compared to the control. Meanwhile, there was a statistically significant ($P < 0.01$) difference in the creatinine level of the 500 mg/kg extract treated group and the control. There was no statistically significant ($P > 0.05$) difference in the level of blood urea and total cholesterol between the treated groups and the control. However, there was a marginal increase which is not statistically significant ($P > 0.05$) in the total protein level of the group administered with 1000 mg/kg of extract and the control

Parameters	Extract (250 mg/kg)	Extract (500 mg/kg)	Extract (1000 mg/kg)	Control (5 ml/kg)
AST (IU/L)	184.20 ± 10.41	184.60 ± 20.87	193.80 ± 9.50	170.40 ± 10.40
ALT (IU/L)	76.80 ± 4.90	72.40 ± 12.51	59.60 ± 10.21	66.20 ± 4.90
ALP (IU/L)	82.60 ± 11.46	72.20 ± 7.33	90.40 ± 14.90	73.00 ± 8.52
Urea (mmol/L)	7.02 ± 0.57	7.10 ± 0.58	6.86 ± 0.43	6.12 ± 0.31
Creatinine (μmol/L)	69.40 ± 5.95	76.00 ± 6.96*	65.00 ± 2.74	61.00 ± 1.87
TC (mmol/L)	2.50 ± 0.05	2.32 ± 0.12	2.44 ± 0.08	2.36 ± 0.68
Total protein (g/L)	55.40 ± 3.40	54.40 ± 0.93	60.00 ± 0.84	54.80 ± 2.13

*$P < 0.1$, $P > 0.05$ Values were not statistically significant ($P > 0.05$) as compared to control. $n = 5$, T-test, $P < 0.05$

Table 6 Effect of the ethanol fruit extract on the relative organ weights of rats

Group	Dose (mg/kg)	Liver	Kidney
Extract	1000	2.43 ± 0.26	0.34 ± 0.02
Extract	500	2.26 ± 0.10	0.30 ± 0.01
	Deionized water	2.39 ± 0.10	0.30 ± 0.02
Extract	250	2.78 ± 0.34	0.32 ± 0.02

Values were not statistically significant ($p > 0.05$) as compared to the control group
Values are expressed as mean ± standard error of mean (S.E.M) of 5 rats treated for 30 days

Conclusion

The study conducted revealed that the ethanol extract of *Harungana madagascariensis* fruit produced no significant difference in the hematological and biochemical parameters except in the creatinine level but have nephrotoxic and hepatotoxic potentials. Hence, prolonged use of this plant extract should be done with caution. Further studies will be carried out to establish the effects of the plant extract on the white blood cells.

Abbreviations

ALP: Alkaline phosphate; ALT: Alanine amino transferase; ANOVA: Analysis of variance; AST: Aspartate amino transferase; HB: Hemoglobin; HCL: Hydrochloric acid; n: Number; PCV: Packed cell volume; QCA: Quantitative coronary angiography; RBC: Red blood cell; SEM: Standard error of mean; TC: Total cholesterol; UK: United Kingdom; UPH: University of Port Harcourt; WBC: White blood cell

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Declarations

We the authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all authors.

Authors' contributions

This work was carried out in collaboration between both authors. OAS designed the study, wrote the protocol, supervised and co-financed the study. BM carried out the study, performed the statistical analysis and co-financed the study. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Written approval of the Research Ethics Committee of the University of Port Harcourt was obtained according to International standard with reference number UPH/R&D/REC/04.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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