

## Research Article

# Haematological and Biochemical Patterns in the Liver Function of a Syrup Made From *Vitex Doniana* Fruit

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**Background:** Using an animal model, this study aimed to investigate the haematological and biochemical parameters of *Vitex doniana* fruit syrup for its attribute to liver function.

**Methodology:** Syrup concentrations of 25–45 mg/kg<sup>bw</sup> were considered on the mice with weights of 25–35 g. Groups of seven with 5 mice in each were conducted. Group 1 mice served as the negative control, and were given access to feed and water. Groups 2 – 6 mice were administered orally with 25, 30, 35, 40, and 45 ml of syrup concentrations in a single dose for 3 days. The automated haematologic analyser determined the haematology of the mice's blood. The *in vivo* antioxidants and biochemical assays were determined by chemical methods, and the histopathology with haematoxylin and eosin stain method.

**Results:** Weight gain of change values from 8.12–9.81 g was recorded. Increase in red blood cells (7.32–7.45 m/cu.mm), white blood cells (4.30–4.35 t/cu.mm), lymphocytes (55.21–54.72%), neutrophils (21.64–12.70%), and PCV (41.04–41.16%) were recorded. Values of the catalase were 50.20–58.21 µM/g, glutathione (23.41–28.34 µM/g), and lipid peroxidation (93.54–106.21 µM/g) compared to the negative control that has values of between 48.37–58.27 µM/g). A decrease in unit value was recorded in the biomarker enzymes of alkaline phosphate that ranged from 91.17 to 85.24 IU/L, aspartate aminotransferase from 58.67 to 31.56 IU/L, and alanine aminotransferase from 58.16 to 43.36 IU/L. Decreased values in total bilirubin, creatinine, cholesterol, and urea were conceived compared with the negative control value. However, an increase in the unit was observed in the total albumin that ranged from 3.16 to 3.38 mg/dL, total protein from 6.42 to 6.74 mg/dL, and uric acid from 7.06 to 5.10 mg/dL) along with the syrup concentrations compared with the negative control of 3.75 mg/dL, 7.12 mg/dL, and 5.21 mg/dL respectively.

**Conclusion:** The study concludes that the syrup has no health implications as it has the potential for blood maintenance, good antioxidant quality, and quality enzyme makers for healthy liver function.

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## Introduction

Compounds that are of vital importance in healthcare that are found in medicinal plants have been reported to have many functions in disease healing (Messaoudi *et al.*, 2023; Benmohamed *et al.*, 2023). Plant remedies and derivatives are employed in managing health challenges in some parts of the world where modern medicine is expensive and not

affordable by many individuals. This practice becomes imperative because the plants are readily available and their health products are reliable and effective in disease treatment. The need to upgrade the resources of traditional plant food in Nigeria is high and it therefore calls for the understanding of the available and easily accessible plants. This however includes the various plant species that are underutilized (Imoisi and Iyasele, 2020) due to the advent of modern medicine or no knowledge about their medicinal values.

It has been previously known that vegetables could be of more importance in the supply of human health with minerals and vitamins. Recent research has shown that vegetables also provide numerous heterogeneous plant chemicals active in human health. Such chemicals are alkaloids, phenols, steroids, tannins, saponins and others (Gonnella *et al.*, 2018; Ikewuchi *et al.*, 2019). These chemicals play vital roles in the maintenance of human health such as the scavenging of free radicals in the body, inhibition of bacteria and viruses, boosting of the immune system and the metabolisms of hormones (Ifeanach and Ogunwa, 2021).

Some potential plant extracts and plant foods may cause toxicity at high dosages and could result in damage to the vital organs in the body. The liver which is one of such organs, needs to be protected from damage even with the kind of foods we eat, hazardous chemicals from the environment, drugs, and alcohol. The liver's significance in the health care of humans is of paramount importance hence without it, many disorders such as lack in detoxification, protein synthesis, maintenance and regulation of homeostasis (Yin *et al.*, 2021). Once these manifests, chemical and nutrient processing will be hampered hence liver will not be able to produce the glycogen, protein, and other products necessary for its normal function (Akharaiyi *et al.*, 2022). The liver distortion and diseases in humans could generate excessive free radicals that will suppress the natural defensive mechanisms of the body. The malfunction of the liver can be studied with biochemical physiology and haematological profiles (Akharaiyi and Okafor, 2021; Chen *et al.*, 2021; Dubiwak *et al.*, 2021).

*Vitex doniana* has been in utilization since ancient times in tropical Africa to address various health conditions (Charles *et al.*, 2018). *V. doniana* is a plant of many values used by the urban and rural communities in Nigeria where it is employed for some food products such as jam, and wine (Imoisi and Iyasele, 2020), vicious syrup as food, minerals, vitamins and nutrients supply (Akharaiyi and Maliki, 2023). *V. doniana* has many useful purposes such as medical importance, food source and furniture making (Bunu *et al.*, 2021). All of these have aspects they play in the treatment of diseases such as diarrhoea, malaria, headache, fever, hypertension and many others Aiwonegbe *et al.*, 2018). The leaves and fruits apart from being medically important, served as food for humans. Several studies on it have shown that the contained bioactive compounds are responsible for its antimicrobial, antioxidant, and anti-inflammatory, among others Rani and Sharma, 2013). *V. doniana* is a known plant to many countries worldwide for its various uses as medicine, food and for making fire. The parts of the plant such as root, fruit, leave and stem bark have been employed in traditional medicine for the healing of various diseases such as constipation, diarrhoea, dysentery, haemorrhoids and several others (Barry *et al.*, 2022).

This study is aimed at the function *V. doniana* syrup could play in liver health using an animal model.

## Materials and Methods

### *Acute toxicity test*

The guidelines described by the World Health Organization for evaluating the safety and efficiency of herbal medicines, (WHO, 2000) and the Organization of Economic Co-operation and Development (OECD, 2010) for testing of chemicals were adopted. Mixed of male and female rats weighing between 25 – 35 g were selected for the study. In the study, 6 groups containing 5 rats each were designed. Group 1 was the control and the rats were dosed with drinking water. The groups 2 – 6 mice were dosed with the syrup concentrations of 25, 30, 35, 40, and 45 ml/kg body weight of mice respectively. The rats were monitored for any symptoms of toxicity and mortality in the groups for 14 days (Lorke, 1982). Probit analysis by Miller and Tainter, (1944) was used to estimate the median lethal dose (LSD) of the syrup.

### *Experimental animals*

Swiss albino mice (*Mus musculus*) with a total number of 35 were selected for the study, were acclimatized for two weeks and during which were allowed to normal rat feed and water. Before the experiment, the mice were starved for 18 hours but conducted according to the NIH guide. Ethical clearance for the research was obtained from the Nigeria National Health Research Ethics Committee and the approval number on the performed experimental procedures is NHREC 08/2016

### *Experimental design*

For the experiment, groups of seven with 5 mice in each were conducted. Group 1 served as the negative control, and was given access to feed and water. Groups 2 – 6 mice were administered orally with the syrup concentrations of 25, 30, 35, 40, and 45 ml in a single dose for 3 days.

### *Determination of haematology*

The method of Dacie and Lewis, (2002) was used for the estimation of the mice's red blood cells, monocytes, white blood cells, neutrophil, eosinophil and pack cell volume (PCV) with the automated haematologic analyzer SYSMEX KX21 (SYSMEX Corporation, Japan). Haemoglobin was estimated with the use of Sahli's Hemoglobincomeyer by standard procedures as described by D'Armour *et al.* (1970).

### *Determination of in-vivo antioxidant*

The liver tissues of the experimental mice were harvested and washed severally with cold 10% saline (w/v). The livers were homogenized with a mixture of cold solution KCL in 1.15% (w/v) and 0.1 M potassium phosphate buffer with a pH of 7.4. The homogenate was spurned at 10000 g for 1 h and thereafter used to measure the levels of enzymatic lipid peroxidation (Catalase) with the method of Cohen *et al.* (1970), non-enzymatic antioxidant system (GSH) with the method of Ellman, (1959) and lipid peroxidation (LPO) with the method of Van Der Sluis, *et al.* (2000) to determine the antioxidant status.

### *Assay for biochemical*

The alkaline phosphate (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were carried out using the criteria of Bergmeyer *et al.* (1986a; b). The total albumin was estimated with the criteria of Dumas *et al.* (1971), bilirubin was by the method described by Watson and Rogers, (1961), the estimation of total protein was by the Biuret method described by Donniger *et al.* (1972), uric acid used the method of Carroll *et al.* (1971), creatinine was estimated using the method of Lusgarten and Wenk, (1972), cholesterol determination was by the criteria of Abel *et al.* (1952) and urea with the method described by Fenech and Tommasini (1952).

### *Histopathology of liver*

From each treatment, about 3 cm pieces of the liver were cut, rinsed in normal saline and dehydrated in grades of alcohol (20% - 100%). After the dehydration, xylene was used to clear traces of alcohol and water from the tissues. They were then impregnated in molten paraffin wax for 1 h at a temperature of 60 °C. The tissues were then embedded with molten paraffin wax and allowed to solidify. They were then sectioned with a microtome (Bright, England) at 5 – 6 µm. The sectioned tissues were floated in a water bath regulated at 35 °C picked with a slide previously robbed with egg albumin and allowed to air-dry. The sectioned tissues on the slides were dewaxed using xylene, hydrated, cleared with xylene, stained with haematoxylin and eosin, mounted with DPX, and allowed to air-dry. The slides were observed under a binocular microscope with a USB camera and photographed to ascertain the level of damage or safety with the syrup.

### *Statistical analysis*

The obtained results were expressed as mean ± standard deviation (SD) and were subjected to a one-way analysis of variance (ANOVA). The least significant difference (LSD) was performed for the pairwise mean comparisons, to determine the significant treatment dose at a 95% level of confidence. Values were then considered statistically significant at ( $P < 0.05$ ).

## **Results**

The oral dose of the syrup at a concentration of between 25 – 100 mg/kg body weight was administered to the mice with weights of between 25 – 35 g. There was no observable change in the neuro-behavioural attitude or clinical pathology and mortality rate in the treated experimental mice for the 21 days of acute toxicity study. These two conditions, made us to suitable choose the median lethal dose ( $LD_{50}$ ) of the syrup to be less than 50 mg/kg<sup>bw</sup> of the syrup. The mice were therefore treated with the syrup at concentrations of between 25 – 45 mg/kg body weight (Table 1).

Dose (mg/kg <sup>bw</sup> )	Mortality rate
25	0
30	0
35	0
40	0
45	0
LD <sub>50</sub>	≥ 50 mg/kg <sup>bw</sup>

**Table 1.** Acute toxicity test of the syrup

For the period of the toxicity test, there was weight gain among the mice in the negative and the syrup-treated group. The weight gain recorded from the negative control group of mice was between 27.15±0.3 – 30.20±0.4, while in the treated groups of mice with 25, 30, 35, 40 and 45 mg/kg<sup>bw</sup> were between 25.11±0.7-26.03±0.5, 27.23±0.5-28.05±0.3, 35.03±1.4-36.23±1.0, 30.17±0.1-31.20±1.5, and 34.12±1.11 gram respectively (Table 2).

Treatments	0 day	5 <sup>th</sup> day	10 <sup>th</sup> day	14 <sup>th</sup> day	Change value (g)
Control (-ve)	27.15±0.3a	28.18±0.1bc	28.24±1.2bc	30.20±0.4 <sup>bc</sup>	8.12±0.14 <sup>bc</sup>
25 mg/kg	25.11±0.7a	25.36±0.2a	25.42±0.3a	26.03±0.5 <sup>a</sup>	7.28±0.12 <sup>b</sup>
30 mg/kg	27.23±0.5a	27.25±0.3bc	27.47±0.6bc	28.05±0.3 <sup>bc</sup>	5.85±0.12 <sup>a</sup>
35 mg/kg	35.03±1.4c	35.16±1.2c	36.18±0.5c	36.23±1.0 <sup>c</sup>	10.19±0.30 <sup>c</sup>
40 mg/kg	30.17±0.1b	30.24±0.6bc	30.27±0.3bc	31.20± 1.5 <sup>bc</sup>	8.70±0.18 <sup>c</sup>
45 mg/kg	34.12±1.1bc	34.12±0.3c	34.16±1.3c	35.02±0.2 <sup>c</sup>	9.81±0.21 <sup>c</sup>

**Table 2.** Average weight gain of mice (g)

Values are Means±SD of three replicate determinations

Values having different superscript from a – c per column are significantly different

The red blood cells in the negative control group were 7.50±0.76 million/cu.mm, while in the mice treated with 25, 30, 35, 40, and 45 mg/kg<sup>bw</sup> of the syrup was increased from 7.32±0.52, >7.34±0.22, >7.36±0.36, >7.45±0.14, and >7.48±0.10 million/cu.mm respectively. Despite this increase, the values obtained were within the standard range of between 7-10

million/cu.mm. Also, in comparison to other parameters, the white blood cell count in the negative control was  $4.36 \pm 0.11$  thousand/cu.mm, but increased from  $4.30 \pm 0.12$  in the treatment with 25 ml to  $4.35 \pm 0.18$  thousand/cu.mm in the treatment with 45 mg/kg<sup>bw</sup>. These obtained results were also within the standard range of between 3-9 thousand/cu.mm. A similar trend in percentage increase was observed for haemoglobin, lymphocyte, monocyte, eosinophil, neutrophil and PCV counts. (Table 3). In these parameters, we could as well not observe the values that were above the permissive level.

Group	RBC (million/cu.mm)	WBC (thousand/cu.mm)	Heamoglobin (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Neutrophil (%)	PCV (%)
Standard	7 - 10	3 - 9	11 - 19	43 - 85	1 - 6	1 - 4	5 - 49	40 - 54
Control (-v)	$7.50 \pm 0.76c$	$4.36 \pm 0.11c$	$11.47 \pm 0.04a$	$54.36 \pm 0.36b$	$2.20 \pm 0.05a$	$2.36 \pm 0.05b$	$12.76 \pm 0.11c$	$41.22 \pm 0.16c$
25 ml	$7.32 \pm 0.52a$	$4.30 \pm 0.12b$	$11.47 \pm 0.44a$	$55.21 \pm 0.38c$	$2.40 \pm 0.14b$	$3.06 \pm 1.15c$	$12.64 \pm 0.04b$	$41.04 \pm 0.12a$
30 ml	$7.34 \pm 0.22a$	$4.27 \pm 0.21a$	$11.47 \pm 0.37a$	$52.32 \pm 0.23a$	$2.42 \pm 0.07b$	$2.34 \pm 0.43b$	$12.45 \pm 0.04a$	$41.08 \pm 0.32a$
35 ml	$7.36 \pm 0.36a$	$4.35 \pm 0.22c$	$11.47 \pm 0.15a$	$52.18 \pm 0.13a$	$2.41 \pm 1.12b$	$2.23 \pm 0.04a$	$12.66 \pm 0.30b$	$41.12 \pm 0.15b$
40 ml	$7.45 \pm 0.14b$	$4.35 \pm 0.15c$	$11.47 \pm 0.12a$	$54.41 \pm 0.24b$	$2.25 \pm 0.06bc$	$2.40 \pm 0.16b$	$12.68 \pm 0.19bc$	$41.13 \pm 0.23b$
45 ml	$7.48 \pm 0.10c$	$4.35 \pm 0.18c$	$11.47 \pm 0.34a$	$54.72 \pm 0.19b$	$2.28 \pm 0.11c$	$2.42 \pm 0.44b$	$12.70 \pm 0.07c$	$41.16 \pm 0.26b$

**Table 3.** Effect of *V. doniana* syrup on haematology of mice

Values are Means $\pm$ SD of three replicate determinations

Values having different superscript from a – c per column are significantly different

On the antioxidant activity of the syrup in the mice, there was no significant difference  $P < 0.05$  in the *in vivo* antioxidant of CAT, GSH and LPO among the negative control and the group of mice dosed with the varied syrup concentrations. In the negative group of mice, a value of  $48.3748.37 \pm 2.68$  was recorded for CAT, while it was  $50.26 \pm 1.05$ ,  $51.38 \pm 2.08$ ,  $54.23 \pm 1.06$ ,  $55.42 \pm 1.40$ , and  $58.27 \pm 1.37$   $\mu$ M/g for the groups of mice dosed with 25 ml, 30 ml, 35 ml 40 ml and 45 ml respectively. In the GSH, the value for the negative control was  $23.41 \pm 2.03$  while, it was  $24.67 \pm 1.52$ ,  $25.43 \pm 1.36$ ,  $25.67 \pm 1.08$ ,  $27.18 \pm 1.64$ , and  $28.34 \pm 1.37$   $\mu$ M/g for the groups of mice dosed with the syrup concentrations of 25, 30, 35, 40, and 45 mg/kg<sup>bw</sup> respectively. The recorded value for the negative group of mice in LPO was  $135.54 \pm 2.17$   $\mu$ M/g. In the groups of mice administered with 25, 30, 35, 40, and 45 mg/kg<sup>bw</sup> was  $120.43 \pm 1.28$ ,  $116.18 \pm 3.16$ ,  $114.22 \pm 1.24$ ,  $110.34 \pm 2.42$  and  $106.21 \pm 1.16$   $\mu$ M/g respectively (Table 4).

Group	CAT $\mu$ M/g	GSH $\mu$ M/g	LPO $\mu$ M/g
Control (-v)	48.37 $\pm$ 2.68 <sup>a</sup>	23.41 $\pm$ 2.03 <sup>a</sup>	93.54 $\pm$ 2.17 <sup>a</sup>
25 mg/kg <sup>bw</sup>	50.26 $\pm$ 1.05 <sup>b</sup>	24.67 $\pm$ 1.52 <sup>a</sup>	120.43 $\pm$ 1.28 <sup>c</sup>
30 mg/kg <sup>bw</sup>	51.38 $\pm$ 2.08 <sup>b</sup>	25.43 $\pm$ 1.36 <sup>b</sup>	116.18 $\pm$ 3.16 <sup>bc</sup>
35 mg/kg <sup>bw</sup>	54.23 $\pm$ 1.06 <sup>bc</sup>	25.67 $\pm$ 1.08 <sup>b</sup>	114.22 $\pm$ 1.24 <sup>c</sup>
40 mg/kg <sup>bw</sup>	55.42 $\pm$ 1.40 <sup>bc</sup>	27.18 $\pm$ 1.64 <sup>c</sup>	110.34 $\pm$ 2.42 <sup>c</sup>
45 mg/kg <sup>bw</sup>	58.27 $\pm$ 1.37 <sup>c</sup>	28.34 $\pm$ 1.37 <sup>c</sup>	106.21 $\pm$ 1.16 <sup>c</sup>

**Table 4.** Effect of *V. doniana* syrup on the *in-vivo* antioxidant system of mice

Values are Means $\pm$ SD of three replicate determinations

Values having different superscript from a – c per column are significantly different

In the syrup-treated group of mice, a decrease in values was observed from the least concentration of 25 mg/g to the highest concentration of 45 mg/kg in alkaline phosphate (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). However, in ALP, we recorded a lesser value of 82.26 $\pm$ 1.34 IU/L in the negative control which was comparable to the value of 85.24 $\pm$ 1.18 IU/L in the 45 mg/kg<sup>bw</sup> syrup treated group of mice. In the ALT and AST, a similar decrease in values from the least syrup concentration to the highest was also recorded. Meanwhile, these decreased values in the single syrup dose on concentration bases were comparable to the negative control group of mice (Table 5). The decrease in values in these biomarkers signifies the evidence of healthcare the syrup could play in liver function. Above all, the values obtained in both the negative and syrup treated groups of mice were within the standard ranges to evaluate the syrup for having good conduct in the body system.

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Standard	30 - 120	7 - 56	8 - 34
Control (-v)	82.26±1.34 <sup>a</sup>	37.16±2.12 <sup>a</sup>	28.41±1.43 <sup>a</sup>
25 mg/kg	91.17±1.42 <sup>c</sup>	58.16±1.23 <sup>bc</sup>	58.67±2.34 <sup>c</sup>
30 mg/kg	88.18±3.15 <sup>bc</sup>	57.15±1.28 <sup>bc</sup>	54.43±2.23 <sup>c</sup>
35 mg/kg	88.32±1.44 <sup>bc</sup>	53.26±1.43 <sup>c</sup>	44.18±1.41 <sup>bc</sup>
40 mg/kg	86.54±2.10 <sup>b</sup>	50.42±2.18 <sup>c</sup>	33.37±1.14 <sup>b</sup>
45 mg/kg	85.24±1.18 <sup>b</sup>	43.36±2.40 <sup>b</sup>	31.56±1.43 <sup>b</sup>

**Table 5.** Effect of *V. doniana* syrup on Biomakers in mice

Values are Means±SD of three replicate determinations

Values having different superscript from a – c per column are significantly different

Total albumin in the negative control group of mice was 3.75±0.22, while it was 3.16±0.24, 3.18±0.05, 3.20±0.12, 3.32±0.21, and 3.38±0.06 mg/dL for the treatment with 25 mg/kg – 45 mg/kg<sup>bw</sup> respectively. Comparing these results with the negative control suggests normal activities in liver function. There were also increased values in total protein from 6.42±0.28 to 6.74±0.23 mg/dL in the syrup-treated group of mice when compared to the negative group. This significant increase in total protein on syrup concentration bases, suggests good measures from hepatic damage which will result in normal liver functions. Decreased values alongside syrup concentrations were observed in bilirubin from 0.36±0.06 to 0.19±0.12 mg/dL, uric acid from 7.06±0.37 to 5.10±0.07 mg/dL, creatinine from 1.52±0.33 to 1.52±0.33 mg/dL, cholesterol from 124.12±0.11 to 113.07±0.52 mg/dL, and urea from 20.14±1.02 to 17.28±0.11 mg/dL (Table 6). These effects in comparison to their respective negative controls, and permissive levels for a normal healthcare system, emphasized the harmless of the syrup on the normal function of the liver.



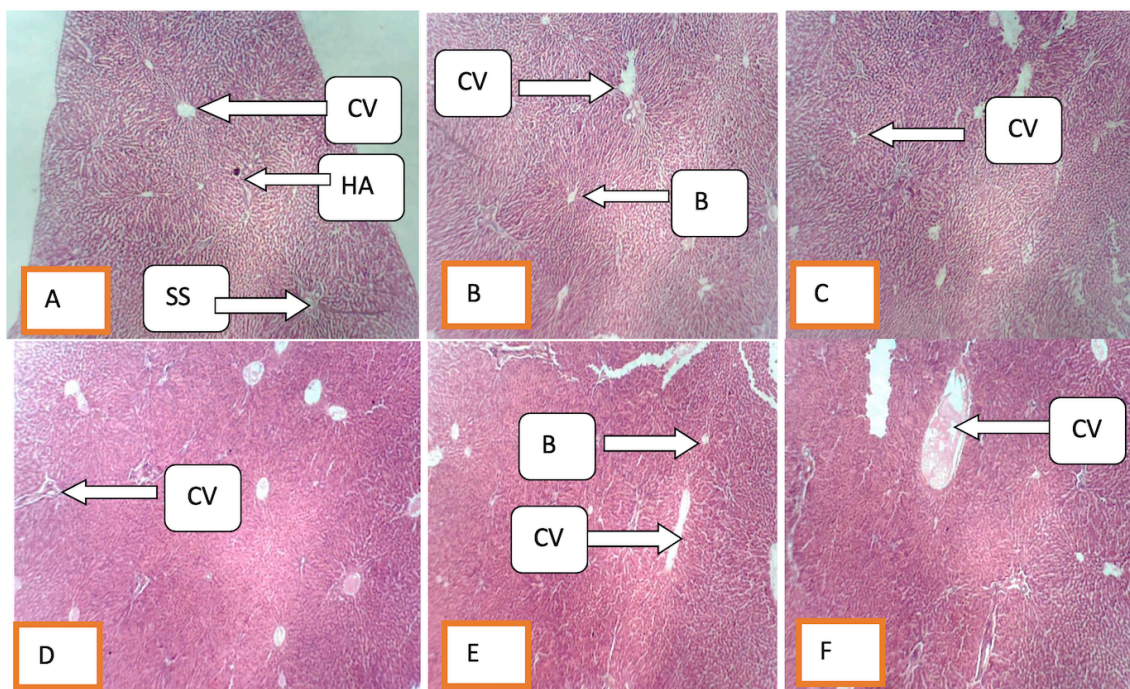
	Total albumin (mg/dL)	Total bilirubin (mg/dL)	Total protein (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	Urea (mg/dL)
Standard	3.5 – 5.3	0.2 – 1.2	6 – 8.3	2.4 – 7.0	0.6 – 1.4	110 – 196	7 – 21
Control (- v)	3.75±0.22 <sup>c</sup>	1.02±0.08 <sup>c</sup>	7.12±0.31 <sup>c</sup>	5.21±0.43 <sup>ab</sup>	1.26±0.21 <sup>a</sup>	109.65±0.35 <sup>a</sup>	16.21±1.22 <sup>a</sup>
25 mg/kg	3.16±0.24 <sup>a</sup>	0.36±0.06 <sup>bc</sup>	6.42±0.28 <sup>a</sup>	7.06±0.37 <sup>c</sup>	1.52±0.33 <sup>c</sup>	124.12±0.11 <sup>c</sup>	20.14±1.02 <sup>c</sup>
30 mg/kg	3.18±0.05 <sup>a</sup>	0.33±0.04 <sup>b</sup>	6.54±0.17 <sup>b</sup>	7.08±0.11 <sup>c</sup>	1.41±0.07 <sup>bc</sup>	120.08±0.34 <sup>c</sup>	20.17±0.31 <sup>c</sup>
35 mg/kg	3.20±0.12 <sup>a</sup>	0.24±0.10 <sup>a</sup>	6.67±0.21 <sup>bc</sup>	6.13±0.16 <sup>bc</sup>	1.45±0.16 <sup>bc</sup>	116.14±0.55 <sup>bc</sup>	19.52±0.22 <sup>b</sup>
40 mg/kg	3.32±0.21 <sup>a</sup>	0.21±0.14 <sup>a</sup>	6.68±0.34 <sup>bc</sup>	5.14±0.21 <sup>a</sup>	1.37±0.10 <sup>b</sup>	114.20±0.12 <sup>b</sup>	18.37±0.04 <sup>a</sup>
45 mg/kg	3.38±0.06 <sup>a</sup>	0.19±0.12 <sup>a</sup>	6.74±0.23 <sup>bc</sup>	5.10±0.07 <sup>a</sup>	1.30±0.23 <sup>a</sup>	113.07±0.52 <sup>b</sup>	17.28±0.11 <sup>a</sup>

**Table 6.** Effect of *V. doniana* syrup in liver functions of mice

Values are Means±SD of three replicate determinations

Values having different superscript from a – c per column are significantly different

The liver function analysis supports the histopathology results as no histological defects such as infiltration, necrotic lesions, and focal necrosis, among other liver distortions were not observed in the representative sectioned liver tissues irrespective of the syrup concentrations. Normal hepatocytes were found around the central vein, the normal architectural structure of well-separated hepatic cells in cord-like arrangement with sinusoids, and bile duct Figure 1).



**Figure 1.** ×100 magnification of liver photomicrograph stained with Hematoxylin and Eosin. A = Mice treated with normal feed and water, B = Mice treated with 25 mg/kg of syrup, C = Mice treated with 30 mg/kg of syrup, D = Mice treated with 35 mg/kg, E = Mice treated with 40 mg/kg, and F = Mice treated with 45 mg/kg. **Legend:** Central vein (CV), Hepatic artery (HA), Bile duct (BD), Sinusoids (SS)

## Discussion

The safety and toxicity of *V. doniana* syrup for its therapeutic use become necessary because it is a plant food with various health benefits that cannot be ignored. The median lethal dose obtained from acute toxicity has been a choice of compliance in choosing safety antimicrobial agent concentrations for reliability study. An active drug must be poisonous, but the level of poison that will not result in death, destruction of organs or disruption of the body system either for short or long-term use is valuable in drug formulations. Evidence for this is the concentrations of an antimicrobial agent that result in death and those of non-mortality after trials on animal models. The median lethal dose of a phytotherapeutic agent is used to determine the minimum dose that will result in 0% mortality and the maximum that will produce 100% lethality (Sha'a *et al.*, 2011). Kennedy *et al.* (1986) have reported that any constituent having LD<sub>50</sub> significant than 5 mg/kg by oral route is non-toxic and therefore safe. The *V. doniana* fruit syrup at 100 mg/kg could not result in any significant effect in the mice pathology for the 21 days of study and for this, there was no concentration(s) to account for 100% death but all in 0%, a dose less than 50 mg/kg was chosen for this research. Imoisi *et al.* (2021), in a study with *V. doniana* syrup concentrations of between 1000 – 5000 mg/kg bw, could not observe any sign of toxicity or death in the mice for their 14-day study. Ayoka *et al.* (2020) have also reported a treatment on rats with doses of 1900, 2600 and 5000 mg/ml and found no weight loss and any abnormal physiological change in the rats for the period of their study. *V. doniana* fruit extract at doses of between 100–300 mg/kg administered to Sprague-Dawley rats in an acute toxicity study,

resulted in no mortality nor signs of neuro-behavioural changes in the treated animals' autonomic nervous system (Adjei *et al.*, 2021). The result of toxicity obtained in this study is an added value to the support of the above reports for *V. doniana* fruit syrup maximum exploit for its economic value.

The weight gain of the mice varies because there was no group of mice with uniform weights. For this, it was not relatively possible to account for significant differences in the weight gains of the mice in the different groups. Dehydration and catabolism of fat and protein often result in weight loss but hence these were not staged in the body system of the mice, there was inhibition of the metabolism of fat and protein for the mice to have weight gain when fed with the syrup for 14 days. The gain in weight of mice in this study is in conformity with the earlier report by Ambiak *et al.* (2013); Ibrahim *et al.* (2014). The weight gain emphasized that the syrup is a quality food that can be absorbed alongside digested food into the body system. *V. doniana* fruit pulp has been reported to contain valuable micronutrients, vitamins and proximate contents that can improve health promotion (Vinchi *et al.*, 2011). The weight gain could also be traced to the young age of the mice hence digestion and absorption are faster in the young an indication of the vital nutrient supply of the syrup.

The blood is a specialized fluid in the body that functions in oxygen supply to cells and tissues, provides essential nutrients to cells, removes waste materials, regulates body temperature, and protects the body from infections. The increased unit values in the red blood cells, haemoglobin, monocyte, neutrophil and PCV counts in haematology analyses, suggest the good effect *V. doniana* has on the health system of the mice. The values obtained were within the standard range of the various parameters analyzed. Asheikh *et al.* (2022) in a study, have also recorded an increase in values on extract concentration bases of Vitex flower leaves fed to goats. *V. doniana* syrup is a plant food with vital constituents which could support and improve immunity which would help maintain a relatively apparent health status. The values of catalase (CAT) and glutathione (GSH) increase on the syrup concentration bases, was not significantly different from the negative control as the range of increase was within standard units. In conjunction with the decreased value alongside syrup concentration observed in the lipid peroxidation (LPO) level, could be certified that the syrup cannot result in free radicals to elicit oxidative stress in the body system. Hence LPO was in decreased values; while CAT and GSH in increased values alongside syrup concentration, emphatically emphasizing that the syrup could have reliable antioxidants to negate hepatotoxicity caused by some chemicals such as paracetamol, carbon tetrachloride (CCl<sub>4</sub>) and others. A similar result was reported by Onoja *et al.* (2014). The relevant factors mostly considered in hepatotoxicity are oxidative stress and LPO (Yousef *et al.*, 2010). Some antioxidants from plants are helpful in free radicals detoxification produced by many stresses (Ouassou *et al.*, 2021). A decrease in GSH measures normally results in endogenous antioxidants known for the suppression of damage from free radicals (Hinson *et al.*, 2010). The envisaged good functions of the in vivo antioxidants in this study could protect the liver from malfunction and attainment of physiological indices which will be termed normal for a healthy system.

The decrease in the ALP, ALT, and AST alongside syrup concentrations found in the circulation of the mice within standard could be subjective to the potential the syrup has on free radical scavenging. These decrease in values was of interest hence were regulations toward the required levels in a normal body system and comparable to the negative control group of mice.

The liver is an important organ in the body known for the detoxification of harmful substances in humans for the maintenance of good health. The food we eat, environmental hazards, chemicals and plant extracts at high dosages can result in liver damage. The selection of substances that will benefit the body and get the liver organ protected at a safe dosage is an important aspect of hepatic study. The increased values in protein and albumin alongside the syrup concentration have a potential for liver protection in functionality and integrity; and to prevent the formation of liver necrosis and lesions. Also, the increase in protein suggests that the liver in its good working condition, was able to link polyribosome with endoplasmic reticulum. The decreased values in bilirubin, uric acid, creatinine, cholesterol, and urea expressed a normal functionality of the liver. With the good status of these parameters, it is predicted that no level of myocardial, hepatic toxicity, or renal damage will be established by the use of *V. doniana* syrup. Other facts in support of this are the low bilirubin, meaning it was able to bind albumin and the radiant nature of albumin hence the liver was able to successfully produce in numbers not below or exceeding the standard level of (3.5-5.3 g/L). These are among the several indices for accuracy in the diagnosis and assessment of risk and therapy adoption (Mohamed *et al.*, 2010). The relation between the unit decrease in urea and creatinine established that this syrup could also alleviate or ameliorate renal dysfunction. The elevation of these parameters in animal studies with a substance indicates rightly the poor condition of kidneys not able to filter them out from the bloodstream. Alkaloid fraction of between 200 – 600 mg/kg concentrations extracted from *V. doniana* showed valuable positive effects on serum protein, lipid profile, and renal function in Wister rats (Ayoka *et al.*, 2023). The liver function analysis supports the histopathology results as no histological defects were observed in the representative sectioned liver tissues irrespective of the syrup concentrations. Normal hepatocytes were found around the central vein, the normal architectural structure of well-separated hepatic cells in cord-like arrangement with sinusoids, and bile duct.

## Statements and Declarations

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### *Ethical Permission*

This research was approved by the Health Research Ethical Committee on the experimental procedure performed on the used mice with the given number NHREC/08/2016.

### *Conflict of interest*

We the authors of this article have no conflict of interest.

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## Authors' Contribution

FCA conceived the research idea, designed and wrote the first draft. CBE managed the literature review, POO performed the analyses. ILE managed literature review and analysis. All authors read and approved the final draft of the manuscript before sending it out for possible publication.

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## Declarations

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