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ORIGINAL ARTICLE

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Evaluation of hematological and biochemical parameters of liver function following consumption of *Vitex doniana* fruit syrup

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ABSTRACT

Background: *Vitex doniana* is a tropical plant whose leaves and fruits are traditionally utilized for medicinal and nutritional purposes.

Aims: This study aimed to evaluate the hematological and biochemical effects of *Vitex doniana* fruit syrup on liver function using an animal model.

Subjects and Methods: Male and female mice with weighing between 24 – 32 g were utilized in this study. Group 1 served as the negative control and was provided with feed and water. Groups 2 – 6 were orally administered single doses of the syrup at concentrations of 25, 30, 35, 40, and 45 mL for 14 days. Hematological parameters were analyzed using an automated hematologic analyzer. *In vivo* antioxidant and biochemical assays were conducted using standard chemical methods, while histopathological assessments were performed using hematoxylin and eosin staining.

Results: The mice showed a weight gain ranging from 8.12 to 9.81 g over the study period. An increase was observed in red blood cell count (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 packed cell volume (PCV) (41.04 – 41.16%). Antioxidant activity showed catalase values between 50.20–58.21 µM/g, glutathione (23.41 – 28.34 µM/g), and lipid peroxidation levels of 93.54 – 106.21 µM/g). Biochemical analysis revealed a reduction in alkaline phosphate (91.17 – 85.24 IU/L), aspartate aminotransferase (58.67 – 31.56 IU/L), and alanine aminotransferase (58.16 to 43.36 IU/L). Decreases were also observed in total bilirubin, creatinine, cholesterol, and urea levels. However, an increase in total albumin (3.16 – 3.38 mg/dL), total protein (6.42 – 6.74 mg/dL), and uric acid (7.06 – 5.10 mg/dL) was recorded with increasing syrup concentrations.

Conclusion: The study concludes that *Vitex doniana* fruit syrup poses no adverse health implications. On the contrary, it exhibits potential for blood maintenance, antioxidant activity, and supports enzymatic functions beneficial for liver health.

Keywords: Antioxidants, biomarkers, hematology, liver function, syrup, *V. doniana*

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1 Introduction

Medicinal plants are known to contain bioactive compounds of significant importance in healthcare with documented roles in the treatment and healing of various diseases (Benmohamed *et al.*, 2023; Messaoudi *et al.*, 2021). In several regions of the world, particularly where modern medicine is prohibitively expensive and inaccessible for large segments of the population, plant-based remedies and their derivatives serve as essential tools for addressing health challenges. In Nigeria, there is a pressing need to enhance the utilization of traditional medicinal resources by identifying and the potential of locally available and easily

accessible plants. This need extends to numerous underutilized plant species (Iyiola & Adegoke, 2023), which remain overlooked either due to the dominance of modern medicine or limited awareness of their medicinal properties.

Several literature sources emphasize the significant role of plants in contributing to human health by providing essential minerals and vitamins. Research has further demonstrated that plants produce diverse bioactive compounds with profound benefits for human health (Gonnella *et al.*, 2018; Ikewuchi *et al.*, 2019). However, certain potential plant extracts and plant-based foods may exhibit toxic effects when consumed in excessive amounts, potentially causing damage to vital organs such as the liver. The liver, being a critical organ, requires protection from damage from damage caused by dietary choices, environmental toxins, hazardous chemicals, drugs, and alcohol. The liver's essential functions in detoxification, protein synthesis, and the maintenance of homeostasis are crucial for overall health (Yin *et al.*, 2021). Impairment of these functions can disrupt chemical and nutrient processing, hindering the production of glycogen, protein, and other compounds necessary for normal physiological function (Akharaiyi *et al.*, 2022). Liver disorders often result in the generation of excessive free radicals, capable of suppressing the natural defensive mechanisms of organism.

The health status and functional integrity of the liver can be assessed through biochemical and hematological profiles (Akharaiyi *et al.*, 2021; Chen *et al.*, 2021; Dubiwak *et al.*, 2021). In this context, *Vitex doniana* has emerged as a plant of significant value in both urban and rural communities in Nigeria. The plant is employed in the production of various food products, including jam, and wine (Iyiola & Adegoke, 2023), vicious syrup as food, minerals, vitamins and nutrients supply (Kamal *et al.*, 2022). The ripe fruit is consumed raw, often as an appetite suppressant, while the plant's leaves and fruits are valued for their medicinal and nutritional properties (Akharaiyi *et al.*, 2023; Bunu, 2021).

Studies have demonstrated that *V. doniana* contains bioactive compounds responsible for its antimicrobial, antioxidant, and anti-inflammatory properties, among others (Aiwonegbe *et al.*, 2018). Various parts of the plant, including its roots, fruits, leaves, and stem bark, are widely employed in traditional medicine for the treatment of conditions such as constipation, diarrhea, dysentery, hemorrhoids and further diseases (Rani & Sharma, 2013; Barry *et al.*, 2022). Notably, Bolanle *et al.*, (2014) reported the hepatoprotective effect of root bark, stem bark, and leaf extracts of *V. doniana* against carbon tetrachloride (CCl_4)-induced liver damage in rats. Additionally, Onwukwe *et al.*, (2020) highlighted the non-toxic profile of *V. doniana* (sweet) leaf fractions in albino rats, underscoring its safety for use in traditional medicine and food products.

This study aimed to analyze the hematological and biochemical effects of *V. doniana* syrup, investigating its potential positive impact on liver health using an animal model.

Despite repeated calls to action, adolescent health remains an underemphasized area of public health concern. There is increasing recognition of the potential of promoting a healthy foundation for future generations by addressing health and nutritional risks among adolescents before pregnancy and parenthood. Adolescence has been identified as a critical window of opportunity to interrupt the intergenerational cycle of malnutrition. This stage provides a unique opportunity to reduce the incidence of health challenges associated with nutritional imbalances by positively influencing dietary behaviors, particularly among female adolescents.

The transformative shift toward sustainable development involves ensuring that female adolescents have access to quality nutrition, equipping them for both productive and reproductive phases of life. However, there is a paucity of data to inform decisions taken regarding potential intervention among adolescents in Nigeria.

Hence, the aim of this study was to assess the dietary proficiency of female adolescents in Nigeria. The null hypotheses tested were: (1) there is no significant difference in the dietary proficiency of in-school adolescent girls across regions, and (2) there is no significant association between sociodemographic characteristics and dietary proficiency among in-school adolescent girls in Nigeria.

2 Material and Methods

2.1 Preparation of *V. doniana* fruit syrup

Ripe fruits of *Vitex doniana* were collected from beneath fruit-bearing trees in the forest. The fruits were carefully sorted and washed with clean water. To prevent seed breakage, the fruits were gently macerated using a pestle and mortar. The resulting pulp was mixed with clean water and filtered through a sieve. The filtrate underwent further purification by passing it through a double layer of muslin cloth to remove any residual debris. The purified filtrate was boiled for several hours until it thickened and developed a blackish color. This thickened, black-colored substance constituted the syrup, characterized by its sweet taste and vicious texture, resembling honey (Akharaiyi *et al.*, 2023).

2.2 Acute toxicity test

The acute toxicity test was conducted in accordance with the guidelines of the World Health Organization (WHO) for evaluating the safety and efficiency of herbal medicines, (WHO, 2000) as well as the Organization of Economic Co-

operation and Development (OECD, 2010). Male and female mice, each weighing between 24 – 32 g, were selected for the study. The animals were divided into six groups of five mice each. Group 1, serving as the control, received only drinking water. Groups 2 – 6 were orally administered with varying syrup concentrations of 25, 30, 35, 40, and 45 mL/kg body weight, respectively. The mice were closely observed for 14 days for any signs of toxicity or mortality within the groups (Lorke, 1983). The median lethal dose (LD_{50}) of the syrup was estimated using the probit analysis method of Miller & Tainter (1994). Meanwhile, the weights of the mice were recorded both before and after the experiment to draw inferences about the syrup's effects.

The LD_{50} of the syrup was calculated using the geometric mean of the lowest causing no mortality and the highest dose resulting in 100% mortality. Formula (1) was applied:

$$LD_{50} = \sqrt{(A \times B)} \quad \ldots\ldots\ldots\ldots (1)$$

Where:

A = the maximum dose of syrup that produces 0% death and B = the dose that produce 100% death rate.

2.3 Animal experimentation

Swiss albino mice (*Mus musculus*), approximately six months old and weighing between 24 – 32 g, were selected for this study. A total of 35 mice, comprising both males and females, were acclimatized for two weeks in a controlled animal house environment. During the acclimatization period, the mice were provided with standard rat feed (Top Feed) and clean water. Prior to the experiment, the mice were fasted for 18 hours. All experimental procedures were conducted in strict compliance with the National Institutes of Health (NIH) guidelines. Ethical approval for the study was obtained from the Nigeria National Health Research Ethics Committee, with the approval reference number NHREC 08/2016

2.4 Experimental design

The study consisted of six groups, each containing five mice. Group 1 served as the negative control and was provided access to standard feed and water. Groups 2 – 6 received single oral doses of syrup concentrations at 25, 30, 35, 40, and 45 mL /kg body weight, respectively, for 14 consecutive days.

The syrup was orally administered using a modified syringe connected to a flexible rubber tube. The rubber tube, attached to the to the syringe containing the syrup, was carefully inserted into the mice's throats to ensure precise delivery. The syringe was then pressed to release the syrup directly into the esophagus. This method ensured accurate dosing and minimized discomfort to the animals.

2.5 Determination of hematological parameters

The hematological parameters, including red blood cell count, monocyte count, white blood cell count, neutrophil count, eosinophil count, and packed cell volume (PCV), were measured using the SYSMEX KX21 automated hematologic analyzer (SYSMEX Corporation, Japan) following the method described by Bain *et al.,* (2000). Hemoglobin concentration was determined using Sahli's Hemoglobinometer as per the standard procedures outlined by D'Armour *et al.*, (1965).

2.6 Determination of *in-vivo* antioxidant activity

Liver tissues from the experimental mice were excised and thoroughly rinsed with cold 10% saline (w/v). The tissues were homogenized in a cold solution containing 1.15% (w/v) potassium chloride (KCl) and 0.1 M potassium phosphate buffer (pH of 7.4). The homogenate was centrifuged at 10000 g for 1 hour and the supernatant was used for biochemical analyses:

- **Catalase (CAT) Activity**: Enzymatic lipid peroxidation was measured following the method of Cohen *et al.* (1970).
- **Glutathione (GSH)**: Non-enzymatic antioxidant levels were determined using the method of Ellman (1959).
- **Lipid Peroxidation (LPO)**: The extent of lipid peroxidation was analyzed using the method described by van der Sluis *et al.* (2000).

2.7 Biochemical assays

Biochemical parameters were determined using established methods as follows:

- **Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT)**: Assessed using the protocols of Bergmeyer *et al.* (1985a, 1985b).
- **Total Albumin:** Measured using the method of Doumas *et al.* (1971).
- **Bilirubin:** Determined using the procedure described by Watson and Rogers (1961).
- **Total Protein:** Analyzed using the Biuret method as outlined by Hutson *et al.* (1972).
- **Uric Acid:** Assessed using the method of Carroll *et al.* (1971).
- Creatinine: Estimated according to the protocol described by Lustgarten and Wenk (1972).

- Cholesterol: Measured using the criteria of Abel *et al.* (1952).
- Urea: Determined using the method described by Fenech and Tommasini (1952).

2.8 Liver histopathology

Liver tissue samples (approximately 3 cm) were excised from each treatment group, rinsed in normal saline, and dehydrated in graded ethanol solutions ranging from 20% to 100%. Following dehydration, residual alcohol and water were cleared from the tissues using xylene. The tissues were then impregnated with molten paraffin wax at 60°C for one hour. After embedding in paraffin wax, the tissues were allowed to solidify before being sectioned into 5–6 µm slices using a microtome (Bright Instruments, England). The sections were floated in a water bath maintained at 35°C, mounted onto slides previously pre-coated with egg albumin, and air-dried. The mounted sections were dewaxed with xylene, hydrated, cleared again with xylene, and stained with hematoxylin and eosin. Finaly, the slides were mounted with Distrene Plasticizer Xylene (DPX), and air-dried. The prepared slides were examined under a binocular microscope equipped with a USB camera. Images were captured to evaluate the extent of tissue damage or safety following syrup administration.

2.9 Statistical analysis

The obtained results were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). Pairwise mean comparisons were conducted using the least significant difference (LSD) test to identify significant differences among treatment doses at a 95% confidence level. Statistical significance was considered at $p < 0.05$.

3 Results

3.1 Acute toxicity characteristics

Oral administration of the syrup at concentration levels ranging from 25 to 45 mg/kg$^{(bw)}$ body weight was administered to the mice weighing between 24 and 32 g. Over the 14 – acute toxicity study period, no observable changes were noted in the neuro-behavioral pattern, clinical pathology, or mortality rates of the treated mice. These findings suggest that the median lethal dose (LD_{50}) of the syrup is less than 50 mg/kg^{bw}. The mice were treated with syrup concentrations from 25 – 45 mg/kg body weight (Table 1).

Table 1. Acute toxicity test of the syrup

Dose (mg/kg^{bw})	Mortality rate
25	0
30	0
35	0
40	0
45	0
LD_{50}	≥ 50 mg/kg^{bw}

Note. bw: body weight

3.2 Weight measurements of mice

During the toxicity test, a general weight gain was observed among the mice in both the negative control group and the syrup-treated groups. The negative control group exhibited a weight gain ranging from 27.15 ± 0.3 to 30.20 ± 0.4. In the syrup-treated groups, weight gains were recorded as follows:

- 25 mg/kg bw: 25.11 ± 0.7g – 26.03 ± 0.5g
- 30 mg/kg bw: 27.23 ± 0.5 g – 28.05 ± 0.3 g
- 35 mg/kg bw: 35.03 ± 1.4 g – 36.23 ± 1.0 g35
- 40 mg/kg bw: 30.17 ± 0.1 g – 31.20 ± 1.5 g
- 45 mg/kg bw: 34.12 ± 1.11 g (Table 2).

3.3 Hematological indices

The red blood cells (RBC) count in the negative control group was 7.50 ± 0.76 million/cu.mm, while an increasing trend was observed in the syrup-treated groups:

- 25 mg/kg bw: 7.32 ± 0.52 million/cu.mm
- 30 mg/kg bw: > 7.34 ± 0.22 million/cu.mm
- 35 mg/kg bw: > 7.36 ± 0.36 million/cu.mm
- 40 mg/kg bw: > 7.45 ± 0.14 million/cu.mm
- 45 mg/kg bw: 7.48 ± 0.10 million/cu.mm.

These RBC values remained within the standard range of 7 – 10 million/cu.mm. Similarly, the white blood cell (WBC) count in the negative control was 4.36 ± 0.11 thousand/cu.mm, while it increased across the syrup-treated groups, ranging from 4.30 ± 0.12 thousand/cu.mm in the 25 mg/kg^{bw} to 4.35 ± 0.18 thousand/cu.mm in the 45 mg/kg^{bw} group. These values also fell within the standard range of 3 – 9 thousand/cu.mm. A similar pattern of increase was observed for hemoglobin, lymphocytes, monocytes, eosinophils, neutrophils, and PCV counts (Table 3). All measured parameters were within their respective permissible ranges:

Table 2. Average weight gain of mice (g)

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

Note. Values are Means ± SD of three replicate determinations
Values having different superscript from a – c per column are significantly different

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

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

Note. Values are Means ± SD of three replicate determinations. PCV: packed cell volume; RBC: red blood cells; WBC: wight blood cells.
Values having different superscript from a – c per column are significantly different

hemoglobin: 11 – 19%; lymphocytes: 43 – 85%; monocyte: 1 – 1%); eosinophil: 1 – 4%; neutrophil: 5 – 49%), and PCV: 40 – 54%.

3.4 *In vivo* antioxidant potential of the syrup

he evaluation of the in vivo antioxidant activity of the syrup in mice at varying concentrations revealed no significant difference ($p > 0.05$) in CAT, GSH and LPO levels between the negative control group and the syrup-treated groups. The negative control group exhibited a CAT value of 48.37 ± 2.68 µM/g was recorded for CAT, whereas the syrup-treated groups showed CAT values ranging from 50.26 ± 1.05 to 58.27 ± 1.37 µM/g for syrup concentrations of 25 to 45 mg/kg body weight (bw). For GSH, the negative control group recorded a value of 23.41 ± 2.03 µM/g, while the syrup-treated groups demonstrated values between 24.67 ± 1.00 and 28.34 ± 1.37 µM/g across the same dose range. The LPO levels for the negative control group were 135.54 ± 2.17 µM/g. Among the syrup-treated groups, LPO values ranged from 106.21 ± 1.16 to 120.43 ± 1.28 µM/g, indicating a dose-dependent reduction in lipid peroxidation (Table 4).

3.5 Biochemical activity of the syrup

In the syrup-treated groups, a dose-dependent decrease in alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels was observed. In

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

Group	CAT (µM/g)	GSH (µM/g)	LPO (µM/g)
Control (-v)	48.37 ± 2.68[a]	23.41 ± 2.03[a]	93.54 ± 2.17[a]
25 mg/kg^{bw}	50.26 ± 1.05[b]	24.67 ± 1.52[a]	120.43 ± 1.28[c]
30 mg/kg^{bw}	51.38 ± 2.08[b]	25.43 ± 1.36[b]	116.18 ± 3.16[bc]
35 mg/kg^{bw}	54.23 ± 1.06[bc]	25.67 ± 1.08[b]	114.22 ± 1.24[c]
40 mg/kg^{bw}	55.42 ± 1.40[bc]	27.18 ± 1.64[c]	110.34 ± 2.42[c]
45 mg/kg^{bw}	58.27 ± 1.37[c]	28.34 ± 1.37[c]	106.21 ± 1.16[c]

Note. Values are Means ± SD of three replicate determinations. bw: body weight; CAT: Catalase; GSH: Glutathione; LPO: Lipid Peroxidation
Values having different superscript from a – c per column are significantly different

particular, the negative control group exhibited an ALP value of 82.26 ± 1.34 IU/L, which was comparable to the value of 85.24 ± 1.18 IU/L recorded in the group treated with 45 mg/kg^{bw} of syrup. Similarly, ALT and AST levels followed a comparable trend, decreasing from the lowest to the highest syrup concentration. Meanwhile, the decrease in these biomarkers suggest potential hepatoprotective effects of the syrup (Table 5). Notably, the values for ALT, AST, and ALP in both the negative control and syrup-treated groups remained within the standard physiological range, underscoring the safe and beneficial influence of the syrup on liver function.

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

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

Note. Values are Means ± SD of three replicate determinations. ALP: Alkaline Phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase
Values having different superscript from a – c per column are significantly different

negative control. This increase in total protein that the syrup may have protective effects against hepatic, supporting normal liver functions.

A dose-dependent decrease was observed in bilirubin levels, from 0.36 ± 0.06 to 0.19 ± 0.12 mg/dL, uric acid levels from 7.06 ± 0.37 to 5.10 ± 0.07 mg/dL, creatinine levels from 1.52 ± 0.33 to 1.52 ± 0.33 mg/dL, cholesterol levels from 124.12 ± 0.11 to 113.07 ± 0.52 mg/dL, and urea levels from 20.14 ± 1.02 to 17.28 ± 0.11 mg/dL (Table 6). When compared to their respective negative controls and the permissible ranges for a healthy physiological state, these results emphasize the

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

Groups	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^{c}	1.02 ± 0.08^{c}	7.12 ± 0.31^{c}	5.21 ± 0.43^{ab}	1.26 ± 0.21^{a}	109.65 ± 0.35^{a}	16.21 ± 1.22^{a}
25 mg/kg	3.16 ± 0.24^{a}	0.36 ± 0.06^{bc}	6.42 ± 0.28^{a}	7.06 ± 0.37^{c}	1.52 ± 0.33^{c}	124.12 ± 0.11^{c}	20.14 ± 1.02^{c}
30 mg/kg	3.18 ± 0.05^{a}	0.33 ± 0.04^{b}	6.54 ± 0.17^{b}	7.08 ± 0.11^{c}	1.41 ± 0.07^{bc}	120.08 ± 0.34^{c}	20.17 ± 0.31^{c}
35 mg/kg	3.20 ± 0.12^{a}	0.24 ± 0.10^{a}	6.67 ± 0.21^{bc}	6.13 ± 0.16^{bc}	1.45 ± 0.16^{bc}	116.14 ± 0.55^{bc}	19.52 ± 0.22^{b}
40 mg/kg	3.32 ± 0.21^{a}	0.21 ± 0.14^{a}	6.68 ± 0.34^{bc}	5.14 ± 0.21^{a}	1.37 ± 0.10^{b}	114.20 ± 0.12^{b}	18.37 ± 0.04^{a}
45 mg/kg	3.38 ± 0.06^{a}	0.19 ± 0.12^{a}	6.74 ± 0.23^{bc}	5.10 ± 0.07^{a}	1.30 ± 0.23^{a}	113.07 ± 0.52^{b}	17.28 ± 0.11^{a}

Note. -values are means± SD of three replicate determination
-values having different superscript a-c per column are significantly different.

3.6 Liver function potential of the syrup

The evaluation of liver function biomarkers revealed that the total albumin level in the negative control group was 3.75 ± 0.22 mg/dL, while the syrup-treated groups exhibited values ranging from 3.16 ± 0.24 to 3.38 ± 0.06 mg/dL across syrup concentrations of 25 mg/kg – 45 mg/kg^{bw}. These results, when compared to the negative control, indicate normal liver function activity. Furthermore, the total protein levels increased significantly, ranging from 6.42 ± 0.28 to 6.74 ± 0.23 mg/dL in the syrup-treated groups compared to the non-toxic nature of the syrup and its potential to support normal liver function without adverse effects.

3.7 Effect of the syrup on liver histopathology

The analysis of liver function was corroborated by the histopathological results. No pathological alterations, including infiltration, necrotic lesions, focal necrosis, or other structural distortions of the liver, were observed in the representative liver tissue sections across all syrup

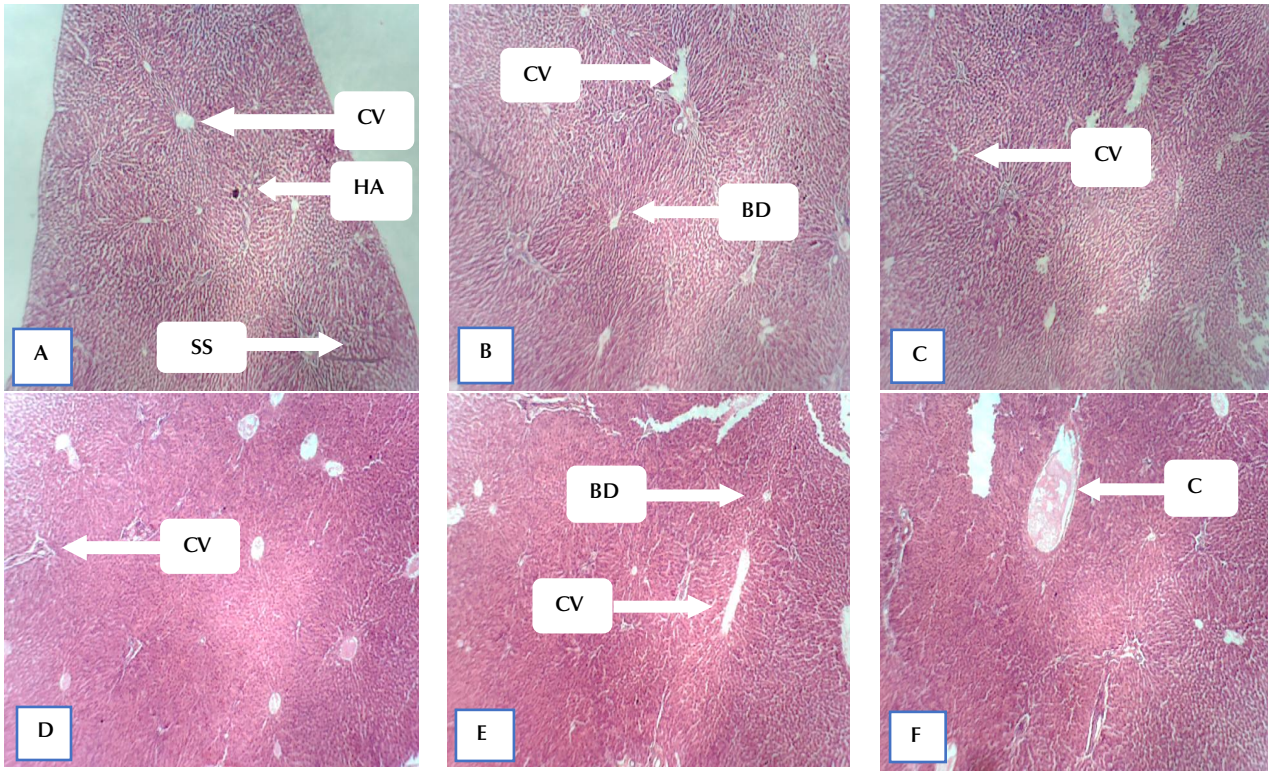

Figure 1. Liver photomicrograph stained with Hematoxylin and Eosin

Note. 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 E: Mice treated with 45 mg/kg. CV: Central vein, Hepatic artery (HA), Bile duct (BD), Sinusoids (SS)

concentration groups. The histological examination revealed normal hepatocytes surrounding the central vein, with an intact architectural arrangement. Hepatic cells were well-organized in a cord-like pattern, separated by clearly defined sinusoids, and a normal bile duct structure (Figure 1).

4 Discussion

Assessing the safety and toxicity of *Vitex doniana* syrup for its therapeutic applications is essential, given its significant health benefits. The median lethal dose (LD_{50}) obtained from acute toxicity testing serves as a critical benchmark for determining safe antimicrobial agent concentrations in reliability studies. While an active drug is inherently toxic to some degree, the importance lies in identifying dosage levels that do not induce mortality, organ damage, or systemic disruption, either in the short or long term. 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 LD_{50} of a phytotherapeutic agent helps establish the range between the minimum dose causing 0% mortality and the maximum dose resulting in 100% lethality (Pillai *et al.*, 2021). Kennedy *et al.*, (1986) reported that any substance with and LD_{50} exceeding 5 mg/kg via the oral route is considered non-toxic and therefore safe. In this study, a maximum concentration of 45 mg/kg of *V. doniana* syrup did not cause any significant pathological effect or mortality in mice during the 14 – day trial. Consequently, no concentration(s) achieved 100% lethality, and doses less below 50 mg/kg were selected for this study. Similar findings have been reported in previous studies. Imoisi *et al.*, (2021) observed no sign of toxicity or mortality in mice administered *V. doniana* syrup at concentrations ranging from 1000 to 5000 mg/kg bw, over 14 – day. Adjei *et al.*, (2021) reported that administering *V. doniana* fruit extract at doses of 100 – 300 mg/kg to Sprague-Dawley rats resulted in no mortality or signs of neurobehavioral alterations in the animals' autonomic nervous system.

Considering these findings, this study employed lower concentrations to further assess the syrup's safety and effectiveness, potentially leading to recommendations for appropriate dosage levels to support safe and effective health

maintenance. The absence of toxicity observed in this study corroborates existing literature, adding to the evidence base supporting the safe use of *V. doniana* syrup. These findings reinforce the potential for maximizing the economic and therapeutic value of this plant-based product.

The variation in weight gain among the experimental mice can be attributed to the initial differences in their baseline weights. For this, it was not relatively possible to account for significant differences in the weight gains of the mice across groups. Weight loss is commonly associated with dehydration and the catabolism of fat and protein. However, as these physiological conditions were not induced in the experimental mice, their metabolic systems exhibited an inhibition of fat and protein catabolism, allowing for weight gain during the 14 – day administration of the syrup. This observation aligns with prior studies by Obasi *et al.*, (2019) and Amuzat *et al.*, (2020), which highlighted weight gain as an indicator of nutritional adequacy. The findings suggest that the syrup is a high-quality food source, capable of being efficiently digested and absorbed alongside other nutrients. *V. doniana* fruit pulp has been documented to contain valuable micronutrients, vitamins and proximate contents that promote health and wellness (Vunchi *et al.*, 2011). Additionally, the weight gain observed may also be attributed to the young age of the mice, as younger organisms tend to exhibit more efficient digestion, further emphasizing the syrup's role as a vital nutrient source.

The blood, a specialized fluid responsible for oxygen transport, nutrient delivery, waste removal, temperature regulation, and immune defense, serves as a key indicator of physiological health. In this study, the increases in red blood cell count, hemoglobin levels, monocyte count, neutrophil count, and PCV values underscore the beneficial effects of *V. doniana* syrup on the mice systemic health. These hematological parameters remained within standard reference ranges. The syrup's antioxidant properties were evidenced by the increased CAT and GSH values which, though not significantly different from the negative control, remained within standard physiological units. In conjunction with the decreased value alongside syrup concentration observed in the LPO levels, this highlights the syrup's inability to elicit oxidative stress through free radical generation. This pattern of increased CAT and GSH alongside decreased LPO suggests that *V. doniana* syrup possesses reliable antioxidant properties that may mitigate hepatotoxicity induced by harmful chemicals such as paracetamol and carbon tetrachloride (CCl_4). These findings are consistent with the results of Onoja *et al.*, (2014), who reported comparable antioxidant effects. Oxidative stress and LPO are critical factors in the onset of hepatotoxicity (Yousef *et al.*, 2010). Plant-derived antioxidant play a significant role in detoxifying free radicals generated during stress (Ouassou *et al.*, 2021). A decrease in GSH compromises the body's endogenous antioxidant defenses, increasing vulnerability to free radical-induced damage (Hinson *et al.*, 2010). However, the robust antioxidant activity observed underscores the syrup's potential to protect the liver, maintain physiological integrity, and support overall health.

The observed decreases in ALP, ALT, and AST levels across syrup concentrations within the standard range may indicate the syrup's potential as a free radical scavenger. These decreases are noteworthy as they suggest regulatory mechanisms that maintain enzyme levels consistent with a normal physiological state, comparable to those of the negative control group of mice.

The liver, a vital organ responsible for detoxifying harmful substances, plays a crucial role in maintaining human health. Factors such as dietary components, environmental toxins, chemicals, and plant extracts at high dosages can lead to liver damage. Identifying substances that protect the liver at safe dosages is a key aspect of hepatic research. In this study, the increase in protein and albumin levels with escalating syrup concentrations highlights the syrup's potential to support liver functionality and integrity while preventing liver necrosis and lesions. Furthermore, elevated protein levels also suggests that the liver, in its optimal condition, successfully facilitated the linkage of polyribosomes with endoplasmic reticulum. Additionally, the decreased values observed in bilirubin, uric acid, creatinine, cholesterol, and urea demonstrate normal liver function. These findings suggest that *V. doniana* syrup does not induce myocardial, hepatic, or renal toxicity. For instance, low bilirubin levels indicate effective albumin binding, and the liver's ability to produce albumin within the standard range (3.5 – 5.3 g/L) reflects its functional integrity. These parameters are among the several indices for accuracy in the diagnosis, risk assessment, and formulating therapeutic strategies (Mohamed *et al.*, 2010).

The noticed decrease in urea and creatinine levels suggests that the syrup may alleviate or prevent renal dysfunction. Elevated levels of these parameters in animal model are often indicative of impaired kidney function and the inability of the organ to filter waste from the bloodstream. Studies on *V. doniana* alkaloid fractions (200 – 600 mg/kg concentrations) showed valuable positive effects on serum protein, lipid profile, and renal function in Wister rats (Ayoka *et al.*, 2023).

Liver function analysis in this study is further supported by histopathological results, which revealed no pathological abnormalities in the sectioned liver tissues, irrespective of the syrup concentration. The preserved normal architecture of hepatocytes, central vein, well-separated hepatic cells arranged in cords with sinusoids, and bile duct underscores the syrup's safety profile.

5 Conclusions

The experimental concentrations of 25 – 45 mg/kg in mice demonstrate that the fruit syrup of *V. doniana* holds significant promise for health maintenance. Based on the hematological and biochemical findings, coupled with the absence of pathological effects in the liver tissues, this study concludes that the syrup poses no adverse health implications. *V. doniana* fruit syrup exhibits potential for maintaining healthy blood parameters, providing antioxidant benefits, supporting liver enzymatic function, and promoting overall systemic health in animal models.

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