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TOXICITY PROFILE OF AQUEOUS LEAVES EXTRACT OF *Vitis vinifera* (PURPLE GRAPES) ON WISTAR ALBINO RATS

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ABSTRACT
Many different diseases have been treated using Vitis vinifera leaves in traditional medicine. Despite their advantages, there haven't been many investigations on their possible toxicity. This work aimed to investigate the acute and sub-acute toxicological effects of Vitis vinifera leaf extract. Its phytochemical test indicated that the plant contains phytochemicals flavonoids, saponins, steroids and triterpenoids. The various classes of phytochemicals in the Vitis vinifera showed the anti-microbial potency of the plant. The acute toxicity of this plant was assessed using Lorke's method. The acute and sub –chronic toxicity tests of Vitis vinifera aqueous extract presented oral LD_{50} of 2828.43mg/kg, at doses of 250, 500 and 1000 mg/ml by examining the general behavior, clinical signs, relative organ weight and histopathology changes. In other words, different plant extract concentrations had no effect on the diseased state of the kidney and liver. Additionally, neither the extract nor any treatment-related changes in body weight, food consumption, or hydration level occurred between the treatment and control groups. The liver and kidney underwent histopathological examination, but no morphological changes were found. The findings of this investigation demonstrated LD_{50} ranges from 10 to 5000 mg/kg body weight, suggesting that the plant's (Vitis vinifera leaves) aqueous extract is non-toxic. The daily oral dose of Vitis vinifera aqueous extract for 28 days demonstrates that it is neither hepatotoxic nor nephrotoxic.
Keywords: phytochemicals, toxicity tests, traditional medicine, Vitis vinifera,

INTRODUCTION

The hunt for solutions to the global problems of antibiotic resistance in pathogenic bacteria has frequently been fueled by the isolation and identification of novel antimicrobial chemicals from a variety of sources, including medicinal plants (Rahman, 2011). This is most likely because these plant products' efficacies have been proven in a range of disease conditions around the world, and they have been successful when most synthetic or conventional medicines have failed because of their minimal or nonexistent adverse effects. It might also be because research has shown that certain plants' pure components and crude extracts can enhance the in vitro action of antibiotics (Orisakwe, 2003).

For a very long time, Africans have employed medicinal preparations to treat illnesses. This is attributable to a variety of factors, including the fact that first-line medications, which are typically inexpensive and accessible, are no longer effective owing to resistance. But as people become more aware of how gently they can be strengthened and how most of them can be taken without the known negative effects of medications, they are starting to use these plant-based medicines more and more (Srinivan, 2012). Higher plants are viewed by scientists that study plant remedies as living chemical factories that produce a variety of strange chemical compounds with a diversity of biological functions. Plants can create substances that, while serving no discernible purpose in the plant's basic metabolism, have strong antibacterial activity when bacterial pathogens are able to penetrate and aggregate in them. These substances have only ever been used as medicinal agents in the past (Ali *et al.*, 2012).

The majority of civilized nations have been preoccupied with the worldwide practice of conventional medicine. Both herbal and conventionally-trained doctors are thinking about using the former in Nigeria, where both types of treatment are used.

Despite the widespread use of traditional methods of healthcare delivery and the accessibility of medicinal plants in our local markets, these methods are rarely, if ever, used in public health facilities. Accordingly, it is claimed that issues arise from a lack of sufficient knowledge of the subject, the toxicity of medicinal herbs, and long-standing traditional medical practices, some of whose medicinal qualities and components have been proven scientifically, while others have not yet been the focus of in-depth scientific research. A common problem with the use of crude extract in ethno medicine is the lack of toxicological evaluation of such plants (Guta, 2007).

Therefore, lack of enough studies on the toxicity profile *Vitis vinifera* plant, prompted the present study, with the view to investigate the acute and sub-acute toxicological effects of *Vitis vinifera* leaf extract.

MATERIALS AND METHODS

Collection, Identification and Authentication of the Plant

The leaves of *Vitis vinifera* was collected in the month of February 2022 from Unguwan Dosa, Kaduna State Nigeria. The plant was identified and authenticated by a botanist at the Department of Biological Sciences Kaduna State University, Kaduna, and a voucher specimen number 091 was deposited.

The leaves were air dried, pulverized and grinded to a fine powder using a mortar and pestle, it was then sieved and stored in an air tight container. The dried powder was used to prepare the extracts.

Methodology

Extraction of the plant material

The maceration method was used to perform aqueous extraction. For three days, 1,500 ml of distilled water was used to rehydrate the powder plant materials (400g). The resulting mixture was concentrated to dryness in a water bath after filtering through Whatman No. 1 filter paper. In order to be used later, the dried extract was stored in a secure bottle.

Toxicology studies used the computed percentage yield after it had been reconstituted with distilled water. The calculation was used to determine the extract's yield percentage.

$$\% \text{ Extraction} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

Phytochemical Screening

The extract was subjected to phytochemical screening of the following bioactive components carbohydrate, anthraquinones, cardiac glycosides, alkaloids, saponins, steroids, terpenoids, tannins and flavonoids, using methods adopted from researchers.

Test of Anthroquinones

An extract sample was agitated with 10 ml of benzene before being filtered. The filtrate was then given a quick stir after receiving 5 ml of a 10% ammonia solution. Anthroquinones are present when a pinkish-red or violet colour is produced (Okerulu, 2001).

Test for Carbohydrate

A few drops of Molish reagent were added to a small amount of extract in a test tube, and a small amount of concentrated sulfuric acid was allowed to run down the side of the test tube. This resulted in the formation of a lower purple to violet colour at the interface, which denotes the presence of carbohydrates (Okerulu, 2001).

Test for Cardiac Glycosides

The Kella–Killani test was used. In glacial acetic acid that may have contained residues of ferric chloride, the extract was dissolved. When a purple ring is present at the interface, cardiac glycosides are present (Chinwe, 2001).

Test for Saponins

Ten ml of pure water was added sparingly for the frothing test. After 30 seconds of vigorous shaking, it is let to stand for 30 minutes. The presence of saponins in the extract is indicated by the formation of a honeycomb after more than 30 minutes (Chinwe, 2001).

Test for Steroids/Terpenoids

In a test tube over a pot of boiling water, around 10ml of the extract was evaporated to dryness. A pipette was used to add 2 ml of concentrated sulfuric acid from the bottom of a dried test tube to the residue (Liebermann-Burchard reaction). A reddish brown or violet brown ring appears to form. This demonstrates the existence of triterpenes and steroids (Okerulu, 2001).

Test for Tannins

The use of lead subacetate was made. The extract was dissolved in a solution, and then three drops of lead sub-acetate solution were added. Tannins are shown by a coloured precipitate (Chinwe, 2001).

Test for Flavonoids

The Shinoda test was used. In the heat, 0.5g of the extract was broken down into 1-2 ml of 50% methanol. Four or five drops of strong hydrochloric acid were added, along with metallic magnesium. The presence of flavonoids is indicated by a red or orange colour (Okerulu, 2001).

Test for Alkaloids

The Mayer test was applied. A few drops of the aforementioned reagent were applied to the extract sample in a test tube (Chinwe, 2001).

TOXICOLOGICAL ASSAY

Source of Experimental Animals

Animals - Adult rats weighing 18 to 25 g were purchased from the Kaduna, Nigeria-based National Institute of Trypanosomiasis and Onchocerciasis Research. Before beginning the experiment, they were housed in a cage for two weeks and given ad libitum access to water and grower's mash (Ultima feed). They were kept in regular humidity, temperature, and 12-hour light/dark cycles.

Animal Grouping

Group 1-Normal control (18-22 g)
Group 2-Treated with 250 mg/kg body weight per day (18-20 g)
Group 3-Treated with 500 mg/kg body weight per day (18-22 g)
Group 4-Treated with 1000 mg/kg body weight per day (18-25 g).

Administration of Extract

Acute toxicity studies (Determination of LD_{50})
Acute toxicity studies was carried out using Lorke's method to determine the LD_{50}, It was carried out in two phases.

First Phase of Lorke

The rats were weighed and divided into three groups of three each using a randomization approach. Their body weight was used to determine how much extract needed to be delivered.

For groups I, II, and III, respectively, the rats received treatment with the aqueous extract of *Vitis vinifera* at doses of 10,100 and 1000 mg/kg body. For 24 hours, the animals were intensively monitored for signs and symptoms of intoxication and demise. Their height, pulse, and body temperature were noted both before and after the extract was administered.

Second Phase of Lorke

The doses for further determining the extracts were selected based on the findings from the experiments' initial phase. The rats were weighed and divided into three groups of one rat in each individual cage at random. At doses of 1600, 2900, and 5000 mg/kg body weight, respectively, the extracts were administered to the rats. They were meticulously watched for 24 hours for indications of drunkenness and demise (OEDC, 2001).

Sub-Chronic Toxicity Studies

In this instance, the rats were weighed and randomly divided into four groups of five rats each. Groups B, C, and D received extract treatments at doses of 250, 500, and 1000 mg/kg body weight, while group A serves as the control. The doses employed in the study represented the 1/3 value of the LD50 and were utilised to obtain the necessary amount of the extract to be delivered during the 28-day period during which the groups' intake of food and drink was tracked. Rats' body temperatures were monitored daily, while weekly body weight measurements were taken. The animals must be examined for physical signs of poisoning and demise. The animals were starved for the entire 29th day before being weighed and dissected. The animals were decapitated after being given a chloroform anaesthesia so they could be dissected. The liver and kidney were separated after the sacrifice and kept in 1% formalin for histopathology research.

Relative Organ Weight and Histopathology

Each animal's kidney and liver were removed (carefully examined for gross pathological changes and weighed).

Absolute organ weight was converted to a proportion of body weight to obtain relative organ weight (ROW). Further samples of the liver and kidney were obtained for histology.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Weight of rat on sacrifice day (g)}} \times 100$$

Histopathology, a combination of the Greek words for "tissue," "suffering," and "study of," is the study of disease symptoms through the microscopic inspection of tissues. Surgery is the first step in the histopathological assessment of the liver and kidneys. The organs were taken out of the body and put in a fixative to stabilize them and stop deterioration. The organs were dehydrated, cleansed, wax-impregnated, and then embedded in paraffin wax, causing a block to develop. The block was trimmed and then mounted onto a rotary microtome for sectioning. The sections were collected on a fixed glass slide. Dewaxing of sections was done by placing the shades on a water bath of temperature 40^0C staining with iron haemotoxylin and eosin were carried out for proper viewing of structures. The sections were mounted on Canada balsam and then covered before viewing under x10, then x40 objectives of the microscope.

RESULTS

The percentage yield of the aqueous extract of *Vitis vinifera*

The physical properties of the aqueous extracts and the yields in terms of percentages of the leaf extracts are presented in Table 1. In general, the extracts had a firm, sticky appearance and seemed dark brown in colour with 34% extraction recovery.

Some Secondary Metabolites of the Aqueous Extract of *Vitis vinifera*

According to the results of phytochemical screening for the bioactive metabolites found in

the aqueous extract of *Vitis vinifera* leaves (Table 2). The leaf extracts showed the presence of carbohydrates, saponins, steroids, terpenoids, flavonoids, alkaloids, and glycosides.

Acute Toxicity Test of the Aqueous Extract on Leaves of *Vitis vinifera*

At the various oral doses of the extract 10, 100, 1000, 1200, 2900, 5000 mg/kg, no death was recorded. In this study, the oral LD_{50} value was estimated to be between 10 – 5000 mg/kg body weight, which indicated that the aqueous extract of the leaves of this plant is practically non-toxic acutely (Lorke ,1983), as shown in Table 3.

Table 1: The Percentage Yield of Aqueous Extraction of *Vitis vinifera*

Extract	% yield of the extract	Colour of the extract	Solvent of Extraction
Vitis vinifera	34.4	Dark brown	Distilled water

Table 2: Secondary Metabolites of the Aqueous Extract from *Vitis vinifera*

Phyto-constituents	Test	Inference
Carbohydrate	Molisch test	+
Anthraquinones	Borntragers	_
Cardiac-glycosides	Kella-Kellani	+
Saponins	Frothing test	+
Terpenoids	LIbermanBuchaid	+
Tannins	Ferric chloride	+
Flavonoids	Sodium hydroxide	+
Alkaloids	Arasendorff	+
Glycosides	Fehling test	+

Key:
+ = Presence of Phytochemicals and - = Absence of Phytochemicals

Table 3: Acute Toxicity Test` of the Aqueous Leaves Extract of *Vitis vinifera*

Oral Doses (mg/kg)	Death/Survival rate (Phase I)
10	0/3
100	0/3
1000	0/3

Oral Doses (mg/kg)	Death/Survival rate (Phase II)
1600	0/1
2900	0/1
5000	0/1

(By determining square root of the two values)

$LD_{50}(oral)$

LD_{50} (Oral) = 5000^2 x 1600

= 2828.43

Relative Organ Weight of the rats treated with *Vitis vinifera* extract

The relative organ weight (ROW) result statistically demonstrated that the mean values of the liver and kidney were similar (P value = 0.05). The average relative organ weights of the treated group's liver and kidney are significantly different from those of the control group, it is found (Table 4). In other words, even while the ROW is within the normal reference value, different dosing concentrations of the plant extract appear to have affected the relative organ weight of the liver and kidney, suggesting that the influence may not be pathologically significant.

Table 4: Relative Organ Weight of the rats treated with *Vitis vinifera* extract

Organ	Control	250	500	1000
Liver	0.05 ± 0.008^a	0.034 ± 0.006^b	0.025 ± 0.006^b	0.026 ± 0.0004^b
Kidney	0.0017 ± 0.00032^b	0.0033 ± 0.009^b	0.0025 ± 0.0008^b	0.0031 ± 0.001^b

Note:
Values are expressed as mean ± SD for N =5, Values with different superscripts across the row are statistically significant, While values with same superscript across the row are not significant
P value = <0.05).

Histopathology of liver and kidney of experimental model treated with *Vitis vinifera* leaves extract

The kidney's tubules and glomerulus were normal, and the control group's liver histology revealed normal hepatocytes. The kidney of the 250-treated group displayed a modest lymphocyte hyperplasia, whereas the liver displayed normal characteristics. The liver treated with 500 mg/kg of the extract had typical histological liver structure and a mild kupfer cell hyperplasia (Figure 4.1 and 4.2). Rats given 1000 mg/kg showed mild tubular deformities and lymphocyte hyperplasia in the kidney along with tubular necrosis.

The result of Histopathology of the rats treated with *Vitis vinifera* aqueous leaves extract

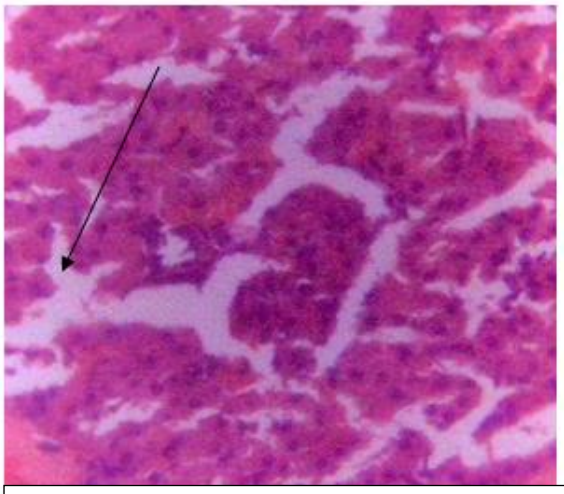


Figure 4.1a: 250mg/Kg kidney showing moderate tubular necrosis

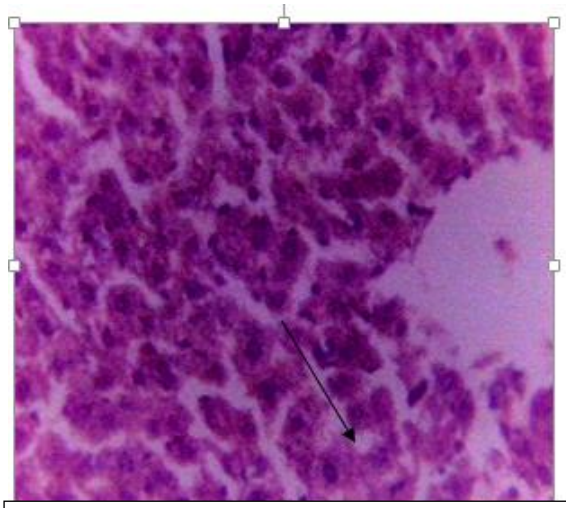
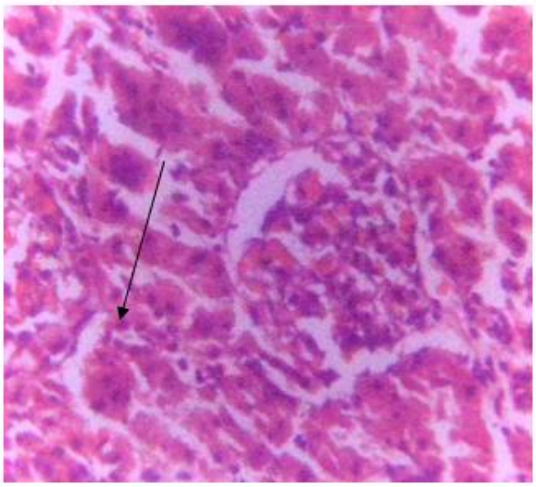
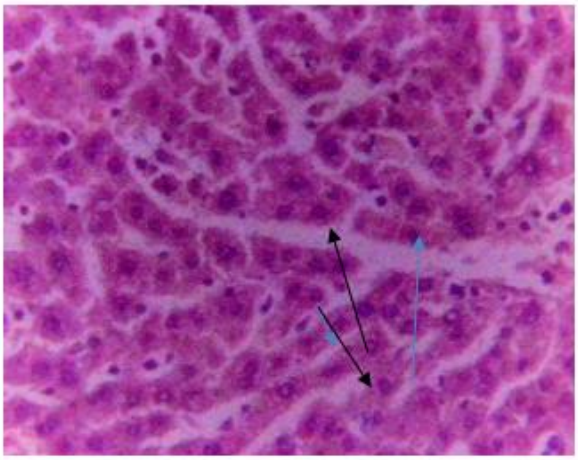


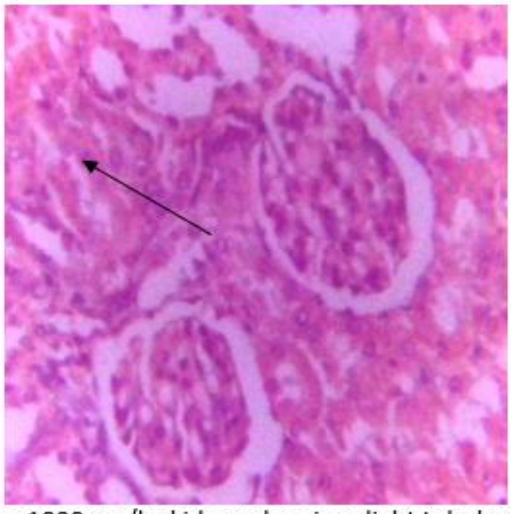
Figure 4.2 b. 250mg/kg liver showing slight hepatic necrosis



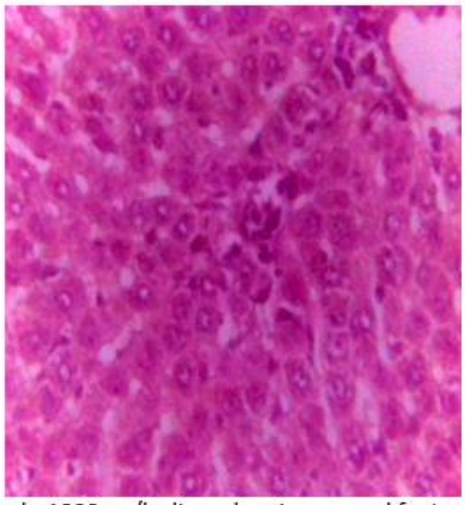
a. 500mg/kg kidney showing moderate tubular necrosis



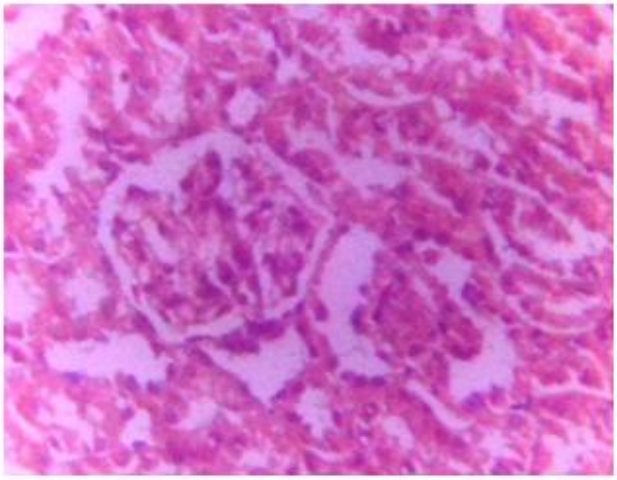
b. 500mg/kg liver showing kupfer cell hyperplasia



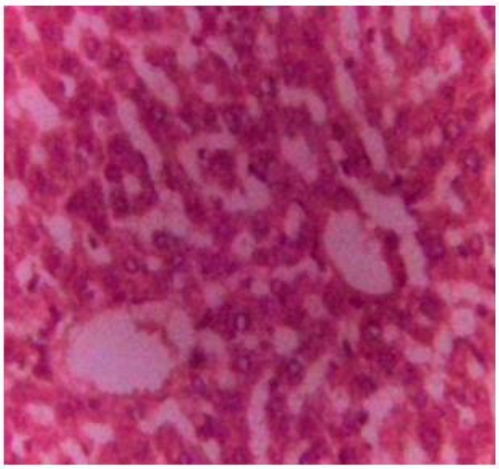
a. 1000 mg/kg kidney showing slight tubular distortion



b. 1000mg/kg liver showing normal feature



a. Control; kidney showing normal feature



b. Control; liver showing normal feature

DISCUSSION

Despite appearing to aid in the prevention and advancement of diseases, medicinal plants and the chemicals generated from them are not nutrition. To make decisions on their useful usage in illness management, it is crucial to be aware of their safety and potential negative effects. In this context, it is necessary to establish scientifically the general toxicity of medicinal plants and their compounds.

The grapevine leaves are composed of a variety of polyphenols which include anthocyanin, flavonoids, tannins and procyanidins (Pandey et al*.,* 2000).

Considering the outcomes of (Table 1). It was established that the yield of the aqueous extract of *Vitis vinifera* was 34.4%. The plant extract is relatively safe when administered and is acceptable for eating because the acute toxicity (LD 50) of the extract is larger than 5000 mg/kg body weight (Table 3).

After oral sub-acute exposure for 28 days in the sub-chronic toxicity trial, no concentration caused treatment-related changes in body weight, food intake, or water level in albino rats (Sharma et al*., 2015*).

The relative organ weight including the liver showed non-significant differences between the control and treatment groups. Also the histopathological examination of the liver and kidney showed no morphological disturbances (Adlin Afzan et al*., 2012*).

All things are deadly and nothing is without poison, according to Paracelsus (The Father of Toxicology), and only the dose prevents something from becoming poisonous (Paracelsus, 2001).

Conclusion

The result of the study clearly indicates that the plant has the presence of carbohydrate, cardiac–glycosides, saponins, terpenoids, tannins, flavonoids, alkaloids, and glycosides. The LD_{50} is between 10-5000 mg/kg body weight at 2828.43 mg/kg. It also indicates that the aqueous extract of the leaves of this plant (*Vitis vinifera* leaves) is practically non–toxic acutely the daily oral administration of the aqueous extract of *Vitis vinifera* for 28 days shows that it is non hepatotoxic and non-nephrotoxic.

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