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ACUTE AND SUB-ACUTE ORAL TOXICITY EVALUATION OF METHANOL LEAF EXTRACT OF *Vitellaria Paradoxa* gaertn F. IN WISTAR RATS

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ABSTRACT

Toxicity studies of medicinal plants are essential for regulatory approval and for their therapeutic application Aim:The study aimed at evaluating the acute and sub-acute toxicity of the methanol leaf extract of *Vitellaria paradoxa* Gaertn F. in Wistar rats.Methods:Phytochemical screening of the extract was done using the methods of Trease and Evans (2004).The LD_{50} was determined using OECD (2008) Guideline 425, while the sub-acute toxicity test was conducted for 14 days at extract doses of 250, 500 and 1000 mg/kg (n=10) using OECD(2008)Guideline407.Carbohydrates,alkaloids,flavonoids,saponins,tannins, steroids/terpenes and cardiac glycosides were found in the extract but anthraquinones were absent. No signs and symptoms of toxicity, and no mortality were recorded in the acute toxicity test and the LD_{50} of the extract was estimated to be greater than 5000 mg/kg. Sub-acute toxicity test of the extract produced significant increases ($p<0.05$) in liver enzymes such as ALT and AST at extract doses tested. All the extract doses significantly decreased ($p<0.05$) serum urea levels, but serum creatinine levels, potassium and chloride ions concentrations were similar to those of the control group. Histological examination of the liver and kidneys showed that the extract produced slight hepatic necrosis and tubular necrosis respectively. All extract doses tested did not significantly ($p>0.05$) alter haematological parameters in Wistar rats.The methanol leaf extract of *Vitellaria paradoxa* is non-toxic in acute toxicity test, but it should be used with caution on prolonged administration as it could be toxic to liver and kidneys.

Keywords: Acute toxicity, Sub-acute toxicity, *Vitellaria paradoxa* methanol leaf extract

INTRODUCTION

It is estimated that about 80% of the people living in developing countries rely on herbal medicines as their primary source of healthcare (WHO, 2008). These herbal medicines are used for the treatment and prevention of diseases and health promotion as well as the enhancement of the span and quality of life (Wachtel-Galor and Benzie, 2011). The use of traditional medicine is not limited to developing countries, and during the past two decades public interest in natural therapies has increased greatly in industrialized countries, with expanding use of ethnobotanicals. In the United States, in 2007, about 38% of adults and 12% of children were using some form of traditional medicine (Ernst *et al*., 2005; Barnes *et al*.,2008). The most common reasons for the use of herbal medicines are: belief of the rural dwellers in their indigenous culture, perception that herbal medicines are natural and safe, accessibility and affordability, long waiting period to see a medical doctor, belief that herbal medicines are more potent than orthodox medicines and self medication as a result of easy availability of plant materials coupled with the little knowledge rural people acquired from generation about health conditions (Okaiyeto and Oguntibeju,2021). Many herbal plants contain bioactive compounds that, while therapeutic at certain levels, can cause adverse effects in higher doses or prolonged use. Toxicity studies help to determine safe usage guidelines, uncover potential side effects, and evaluate any long-term health impacts. Despite their natural origin, herbal remedies can lead to toxicity in major organs, such as the liver and kidneys, or produce allergic reactions and gastrointestinal disturbances. Documenting these toxic effects is essential, as it bridges traditional use with scientific validation, helping to promote safe and effective use in modern healthcare (Bamigbade *et. al.*,2018).

Vitellaria paradoxa, commonly known as the shea tree, is a widely distributed plant in West Africa, renowned for its edible seeds and medicinal properties. The leaves of the shea tree have been traditionally used in folk medicine for various purposes, including treatment of gastrointestinal disorders and wound healing (Adeyemi *et.al*.,2022). The plant has been reported to possess various pharmacological activities, including anti-inflammatory, anti-microbial, and antioxidant effects (Salin *et. al.,2021* Ogunlakin *et. al*.,2023).

Acute toxicity studies on *Vitellaria paradoxa* extracts, have shown minimal adverse effects but acute toxicity tests alone are insufficient to capture the possible risks of prolonged or repeated exposure, as chronic toxicity may manifest in different ways, impacting liver function, immune response, or metabolic health over time. Sub-acute and chronic toxicity studies on *Vitellaria paradoxa* are less common, representing a significant gap in the literature. While some studies have begun to explore the effects of prolonged exposure, the results are not comprehensive or conclusive (McLean, 2015).

Toxicity studies are essential to establish the safety profile of herbal medicines and provide guidance for their rational use (Ekor *et. al*., 2020). The effects of repeated exposure to the extract over a prolonged period of time (14 days) will be assessed in the sub-acute toxicity study in Wistar rats and the Null hypothesis that the methanol leaf extract of *Vitellaria paradoxa* does not cause liver and kidney damage and it does not negatively alter biochemical and haematological parameters in Wistar rats will be tested in this study at extract doses of 250, 500 and 1000 mg/kg (highest dose is 20% of LD_{50} obtained from acute toxicity test)

MATERIALS AND METHODS

Materials

Experimental Animals

Healthy adult Wistar rats of both sexes weighing between 140 and 220g obtained from the Animal House facility of the Department of Pharmacology and Toxicology, Kaduna State University, Kaduna, were used for this study. All animals were kept in clean dry plastic cages and maintained in a well ventilated room in the Animal House at room temperature (25 ± 1°c) and a 12-hour dark/light cycle. The animals were fed with standard feeds and water provided *ad libitum*. All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 83-23 revised1996). All efforts were made to minimize the number of rats used and their suffering.

Plant Material: Vitellaria Paradoxa

The leaves of *Vitellaria paradoxa* were collected in June, 2024 in Zaria, Zaria Local Government Area of Kaduna State. Nigeria. It was identified and authenticated by Mallam Umar Gallah, a taxonomist in the Department of Biological Sciences, Kaduna State University, Kaduna by comparing it with a voucher number 900072 of the Herbarium Numbering System of the Herbarium Unit of the Department of Biological Sciences, Kaduna State University, Kaduna where a copy of the Voucher specimen was deposited for further reference.

Reagents and Chemicals

Dragendoff's reagent (BDH, Poole Ltd, U.K), Concentrated Sulphuric acid, Concentrated Hydrochloric acid, Lead sub-acetate solution, Chloroform, 10% Ammonium solution, Glacial Acetic Acid containing traces of ferric chloride, 10% Sodium hydroxide, Ferric chloride solution, Acetic anhydride, 1% aqueous hydrochloric acid, Molisch's reagent (BDH, Poole Ltd, U.K), Wagner's reagent (BDH, Poole Ltd, U.K), Meyer's reagent (BDH, Poole Ltd, U.K), Methanol (JHD, China), Sodium Hydroxide 140 mmol, Phosphate buffer pH 8.0 100 mmol, Sodium salicylate 80 mmol/l, Sodium nitroprusside 6.0 mmol/l, EDTA, Hematoxylin and Eosin (H&E) stain and alcohol (absolute, 95%, 70%), Normal saline (0.9%, Dana Pharmaceuticals, Minna), Commercial kit for serum liver enzyme assays (Randox Laboratory, England)

Equipment and Apparatus

Avery weighing balance (W and T, Avery Ltd, Birmingham, England), Animal cages , Ceramic mortar and pestle, funnels and filter paper, Glass rod, Conical flasks, Measuring cylinders, Mechanical shaker, Microhaematocrit reader (Hawskey), Rotary Evaporator (Searchtech Instruments, England, RE 52-3), Electric suction pump (Searchtech Instruments, England, RE 52-3) Dry Oven (DHG-9030, Searchtech Instruments, England, RE 52-3), Sterile Syringes and Needles, Mixer, Whatman Filter Paper No 1, Stopwatch, Digital Weighing Balance, Animal Cages, Markers, Water Bath (Model DK-420, No L-606382).

Methods

Preparation of Plant Extract

The leaves collected were air-dried under shade, size reduced using mortar and pestle. It was then ground into a fine powder using a mechanical grinder (Binatone). 250 g of the powdered plant material was macerated in 2.5L of 96% methanol concentration at room temperature for 72 hours with occasional stirring. The liquid was then strained off and the solid residue (marc) was pressed to remove the solution as much as possible. Filtration was carried out using clean muslin cloth and then with Whatman number one filter paper. The extract was concentrated in a Rotary Evaporator (Searchtech Instrument RE 52-3) and finally dried in an oven for 5 days at a temperature of 50^{o} c under reduced pressure. The dried extract was weighed, then kept at 4°c in a refrigerator in an amber air-tight container until required for use. The percentage yield was calculated as:

$$\frac{\text{Weight of dried extract (g)}}{\text{Weight of ground plant material(g)}} \text{x} 100$$

Phytochemical Screening of Extract

The methods of Trease and Evans (2004) were used to screen for the presence or absence of alkaloids, flavonoids, saponins, tannins, glycosides, steroids, triterpenes, anthraquinones and carbohydrates in the methanol extract of *Vitellaria paradoxa.*

Test for Alkaloids

Mayer's test: A small portion of the plant extract was dissolved in 2ml of distilled water, shaken and filtered. few drops of Mayer's reagent were added to the filtrate. Formation of cream-colored precipitate indicates the presence of alkaloids.

Test for Flavonoids

Ferric chloride test: Two drops of ferric chloride were added to the sample solution in distilled water. A greenish colouration is an indication of the presence of flavonoids

Test for Saponins

Frothing Test: 10 ml of distilled water was added to a small portion of the extract in a test tube and was shaken vigorously for 30 seconds. The solution was allowed to stand in a vertical position and observed for 5 minutes. A persistent honeycomb froth is an indication of the presence of saponins

Test for Carbohydrates

Molisch's Test: 3 drops of Molisch's reagent were added to a small portion of the extract in a test tube followed by concentrated sulphuric acid. A reddish coloured ring at the interphase indicates the presence of carbohydrates.

Test for Terpenes/Sterols

Libermann-Burchard's Test: To 0.5 g of the extract, 1 ml of acetic anhydride was added and then dissolved in 1 ml of chloroform. 1 ml of concentrated sulphuric acid was added gently by the side of the test tube to form a lower layer. The formation of a reddish pink or brown ring at the interphase and a bluish green or violet coloured upper layer indicates the presence of steroids and or triterpenes

Test for Tannins

Lead Sub-acetate Test: Small sample of the extract was dissolved in distilled water in a test tube, then 4 drops of lead sub acetate were added to the solution. Cream coloured precipitate indicates the presence of tannins.

Test for Cardiac Glycosides

Keller Killiani's Test: A small portion of the extract was dissolved in 1 ml of glacial acetic acid containing traces of ferric chloride solution. This was followed by the addition of equal volume of sulphuric acid. Appearance of a brown ring at the interphase and a pale green upper layer is an indication of the presence of cardiac glycosides

Test for Anthraquinones
Bontrager's Test: To 2 ml solution of the extract, 5 ml of chloroform was added and the mixture was shaken for 5 minutes. The mixture was then filtered and to the filtrate was added equal volume of 10% ammonia solution with continuous shaking. A bright pink colour in the aqueous upper layer is an indication of the presence of free anthraquinones.

Acute Toxicity Testing
The acute toxicity test was carried out using the OECD (2008) Guideline 425 limit test method. Three female nulliparous Wistar rats (140 – 220g) were used for the study because they are said to be more sensitive than male Wistar rats. The dose used for the limit test was 5000 mg/kg. The test was carried out using the oral route of administration. One Wistar rat was starved of food but not water, overnight. The rat was then weighed after which it was administered the extract at a dose of 5000 mg/kg of fasted body weight orally. Food was then withheld for another 3-4 hours. It was observed for signs and symptoms of toxicity (sedation, vomiting, diarrhoea, salivation, hyperactivity, piloerection) and mortality for 24 hours. Since the rat survived in the first phase, two additional rats were then dosed at the same limit test dose of 5000 mg//kg body weight orally. The rats were observed for signs of toxicity and mortality for 24hours. The two rats survived and limit test was terminated. All tested rats were then observed for 14 days without further dosing with the extract.

Sub-Acute Toxicity Testing
The sub-acute toxicity test was carried out using the OECD (2008) Guideline 407 method. Forty (40) Wistar rats of both sexes (20 males and 20 females) were weighed (140g -220g) and randomized into 4 groups of 10 rats each (5 males and 5 females kept in separate cages). Negative control group rats (Group 1) were treated orally with distilled water (1 ml/kg), while the three test groups (Groups 2, 3 and 4) were treated daily with 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight of methanol extract of *V. paradoxa* orally respectively for 14 days.
Prior to sacrifice on the 15th day of the experiment, the animals were deprived of food for 12 hours overnight on the 14th day. They were then anesthetized using halothane. A portion of the blood samples was collected into EDTA bottles and another portion of blood sample was collected into plain bottles through retro-orbital bleeding.

Biochemical Studies
Blood samples collected from the sacrificed rats into plain bottles were allowed to clot and centrifuged at 3,500 rpm for 10 minutes. The separated sera were stored at - 4ºc, and used for the evaluation of serum liver enzymes which include: alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP), total protein and albumin were measured spectrophotometrically (UV/VIS Spectrophotometer, 752s). Serum urea, creatinine and bilirubin were determined by enzymatic colorimetric methods using Dialab GmbH Diagnostic kit (Catalogue numbers: 402999 Standard for Urea and DO6420 for Creatinine).

Haematological Studies
The portion of blood samples collected into EDTA heparinized bottles were used for estimation of Packed Cell Volume, Red Blood Cells (RBCs) count, Haemoglobin concentration (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cells (WBCs) count and differentials and Platelet count using an automated haematological machine (Cell-Dyn TM Abbot, US).

Histological Studies of Kidneys and Liver
The animals were thereafter sacrificed by cervical dislocation. The liver and kidney were carefully excised and blotted with filter paper to remove traces of blood and perfused with KCl solution (1.15 %). The organs were examined macroscopically and microscopically for morphological changes. The excised liver and kidney samples were fixed in neutral buffered formalin (10 %) prior to histopathological examination. Tissue samples from the kidney and liver of negative control and extract treated rats were then removed and fixed in 10% formalin. Slices of tissues measuring about 5-6 µm thickness were cut off and put in an automatic tissue processor and further fixed in 10% formol-saline solution for 2 hours. Samples were dehydrated for two hours in each of ascending grades of alcohol 70%, 90% and 100% v/v) and then dehydrated in 70%, 90% and 100% alcohol for 1 hour. The dehydrated tissues were cleared in toluene for two hours and tissue slices were embedded in paraffin wax and left to cool. Blocks were trimmed in microtome at microns and ribbon sections floated in a warm water bath. Suitable sections were then dewaxed in xylene and rehydrated in descending order grades of alcohol (100%, 90% and 70% v/v). Sections were then stained in haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in Scott's tap water and stained in eosin for 3 minutes. The sections were then rinsed and dehydrated in ascending grades of alcohol (70%, 90% and 100% v/v). Finally, the sections were cleared in xylene and mounted in a box. They were examined microscopically for pathological lesions. The lesions were observed for the following: infiltration of lymphocytes into portal and central veins, presence of inflammatory cells on the wall, eosinophils, lymphocytes.
Photomicrographs were taken at x 400 magnification.

Statistical Analysis
The results were presented as the means ± standard error of the mean. The data was first subjected to normality test before One-way Analysis of Variance (ANOVA) was performed. Where the result of ANOVA showed that there was at least one inequality between a pair of means, Newman-keuls multiple comparison test, using Graphpad prism version 5.00 (2007) was carried out to locate the position of inequality in means. $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION
Yield of Methanol Leaf Extract of *Vitellaria Paradoxa*
The macerated 250 g of the powdered leaf of *Vitellaria paradoxa* yielded 7.6 g dried methanol extract (3.04 %)

Phytochemical Screening
Preliminary phytochemical screening of *V. paradoxa* methanol leaf extract revealed the presence of carbohydrate, flavonoids, alkaloids, tannins, saponins, cardiac glycosides and steroids/triterpenes. Anthraquinones were not detected in the extract (Table 1).

Table 1: Phytochemical Constituents of Methanol Leaf Extract of *Vitellaria Paradoxa*

Phytochemical Constituents	Test	Observations	Inference
Alkaloid	(Mayer's test)	Formation of cream color precipitate	+
Flavonoids	(Ferric chloride test)	Greenish precipitate	+
Saponins	(Frothing test)	Occurrence of a persistent honeycomb froth	+
Carbohydrates	(Molisch's test)	Reddish coloured ring at the interphase	+
Terpenes/steroids	(Libermann-Burchard's test)	Brown ring at the interphase and a bluish green or violet coloured upper layer	+
Tannins	Lead sub-acetate test	Cream coloured precipitate observed	+
Cardiac glycosides	Keller Killiani test	Brown colour at the interphase and a pale green colour at the upper layer	+
Anthraquinones	(Bontrager's test)	No formation of bright pink color in the aqueous upper layer	-

KEY:
+ = Present - = Absent

Acute Toxicity Testing

The oral LD_{50} of the methanol leaf extract of *Vitellaria paradoxa* was found to be greater than 5000 mg/kg. There were no signs and symptoms of toxicity such as sedation, vomiting, diarrhoea, hyperactivity, muscle twitching (fasciculation) or piloerection and no mortality was recorded.

Sub-Acute Toxicity Testing - Biochemical Studies

Effect of Methanol Leaf Extract of Vitellaria Paradoxa on Liver Function Parameters in Wistar Rats

The level of liver enzymes and proteins is an important parameter for assessing liver health status and the level of its toxicity. Both alanine amino transferase (ALT) and aspartate aminotransferase (AST) showed statistically insignificant increases ($p>0.05$) at 250 and 500 mg/kg doses of methanol leaf extract of *Vitellaria paradoxa* (VPME) when compared with the negative control group (normal saline) but the increase became almost 2 folds ($p<0.05$) at the highest dose of 1000 mg/kg of the extract. There were no significant differences ($p>0.05$) in serum ALT and AST levels among the different doses of the extract tested. There were also no significant changes in the levels of ALP and albumin (($P = 0.655$ and 0.928 respectively) in all the extract treated groups when compared with the negative control group. However, there were significant increases ($P=0.016$) in total protein (TP) levels in the groups of rats treated with 500 and 1000 mg/kg doses of VPME when compared with the negative control (normal saline)

Table 2: Effect of 14-day Oral Administration Methanol Leaf Extract of *Vitellaria Paradoxa* on Liver Function Parameters in Wistar Rats

Liver Enzymes	Normal Saline	VPME 250 mg/kg	VPME 500 mg/kg	VPME 1000 mg/kg
ALT (iu/L)	7.67 ± 1.15^{a}	11.33 ± 2.08^{ab}	12.33 ± 3.06^{ab}	14.33 ± 3.25^{b}
AST (iu/L)	54.00 ± 8.66^{a}	112.00 ± 50.71^{ab}	96.67 ± 11.24^{ab}	167.00 ± 46.03^{b}
ALP (iu/L)	21.17 ± 10.26^{a}	16.67 ± 0.42^{a}	23.87 ± 7.16^{a}	19.20 ± 6.47^{a}
TP (g/dL)	6.40 ± 2.46^{a}	5.87 ± 1.80^{a}	$8.23\pm1.36a^{c}$	11.47 ± 0.90^{c}
ALB (g/dL)	1.57 ± 0.68^{a}	2.00 ± 0.40^{a}	1.43 ± 0.32^{a}	1.73 ± 2.03^{a}

Values are Means ± Standard Deviation. Mean (s) with the same super script in each row are not
significantly different at 5% level of significance ($P>0.05$). ALT= Alanine aminotransferase, AST=Aspartate aminotransferase. ALP= Alkaline phosphatase and TP =Total Protein.

Effects of Methanol Leaf Extract of Vitellaria Paradoxa on Renal, Electrolyte and Oxidative Stress Parameters

The kidney parameters help evaluate the kidney's ability to filter waste, regulate fluid balance, and maintain electrolyte homeostasis. Urea levels decreased significantly in all the extract treated groups compared to normal saline but there were no significant differences in urea levels between 250 and 500 mg/kg doses of the extract tested ($P = 0.07$). The 1000 mg/kg dose of the extract produced significantly lower ($p<0.05$) serum urea levels when compared with the control. Creatinine levels were similar ($P=0.86$) in all extract treated groups when compared with the control (normal saline). There were also no significant changes in potassium ion, chloride ion and bicarbonate levels in 250, 500 and 1000 mg/kg doses of the extract when compared with control ($P>0.05$). Superoxide dismutase level decreased progressively as the dose of the extract increased from 250 mg/kg to 5000 mg/kg but this decrease only became significant at the highest dose of 1000 mg/kg ($p<0.05$) (Table 3)

Table 3: Effect of 14-Days Oral Administration of Methanol Leaf Extract of *Vitellaria Paradoxa* on Kidney Function Parameters in Wistar Rats

Parameters	Normal Saline	VPME 250mg/kg	VPME 500mg/kg	VPME 1000mg/kg
Urea (mg/dL)	31.03±10.58[a]	12.90±8.15[b]	12.50±8.35[b]	24.87±5.64[c]
SOD (mmol/L)	472.8 0± 143.47[a]	433.50 ± 115.94[a]	414.83 ± 94.92[a]	276.27 ± 49.09[b]
K^+ (mmol/L)	4.37 ± 1.29[a]	8.83 ± 7.94[a]	11.87 ± 10.9[a]	12.73 ± 2.39[a]
Creatinine (meq/L)	1.00 ± 0.10[a]	1 .00± 0.61[a]	0.80 ± 0.10[a]	0.90 ± 0.20[a]
Cl^- (mg/dL)	28.00 ± 3.61[a]	38.67 ± 8.39[a]	34.00 ± 7.94[a]	38.67 ± 13.43[a]
Bicarbonate (mg/dL)	92.67 ± 14.43[a]	96.67 ± 27.50[a]	85.00 ± 5.29[a]	82.33 ± 11.59[a]

Values are Means ± Standard Deviation. Mean (s) with the same super script in each row are not significant different at 5% level of significance (P>0.05). SOD=Sodium Dismutase, K^+ = Potassium ion concentration, Cl^- = Chloride ion concentration

Effect of Methanol Leaf Extract of Vitellaria Paradoxa on Haematological Parameters in Wistar Rats

Hematological parameters help assess the immune system and blood cell function. The White blood cells (WBC) play a vital role in immune defense against infections. WBC count increased slightly in all VPME-treated groups compared to the normal saline group, but these increases were not statistically significant (P = 0.31). There were also no significant changes in the lymphocytes, or granulocytes (p>0.05) in the extract treated groups when compared to the negative control group (Table 4). The mid cell count (MID#) was significantly lower (p<0.05) at 500 mg/kg dose of the extract which could be as a result of measurement variation.

The RBC values help assess oxygen transport, anaemia risk, and overall blood health. The extract had no significant effects on RBC count, hemoglobin concentration, hematocrit, or other red blood cell indices when compared with the negative control group (p>0.05) (Table 5).

Platelets are crucial for blood clotting, and variations in these parameters can indicate bleeding disorders, thrombocytopenia, or platelet activation. There were no significant changes in platelet count, size, distribution, or activity in all the extract tested doses when compared with the control group (normal saline) (p>0.05). (Table 6).

Table 4: Effect of 14-Days Oral Administration of Methanol Leaf Extract of *Vitellaria Paradoxa* on White Blood Cell Indices in Wistar Rats

Parameters	Normal Saline	VPME 250mg/kg	VPME 500mg/kg	VPME 1000mg/kg	P. Value
WBC	3.43 ± 0.42[a]	4.90 ± 1.00[a]	4.97 ± 1.62[a]	4.9 0± 1.00[a]	0.31
LYMPH#	6.13 ± 0.21[a]	5.73 ± 0.59[a]	6.57 ± 0.38[a]	5.93 ± 0.93[a]	0.41
MID#	0.83 ± 0.65[a]	1.07 ± 0.67[a]	0.27 ± 0.06[b]	1.07 ± 0.67[a]	0.34
GRAN#	2.63 ± 0.25[a]	3.07 ± 0.15[a]	2.73 ± 0.42[a]	2.87 ± 0.25[a]	0.34
LYMPH%	62.63 ± 5.95[a]	63.5 ± 4.4[a]	65.57 ± 5.41[a]	64.07 ± 5.26[a]	0.92
MID%	3.27 ± 1.45[a]	4.27 ± 0.84[a]	3.73 ± 1.67[a]	3.30 ± 1.50[a]	0.8
GRAN%	34.83 ± 6.5[a]	33.77 ± 6.29[a]	30.8 ± 4.35[a]	33.5 ± 6.62[a]	0.86

Values are Means ± Standard Deviation. Mean (s) with the same super script in each row are not significant different at 5% level of significance (P>0.05). WBC: White Blood Cell count, LYMPH#: Absolute Lymphocyte count, MID#: Absolute Mid cell count (typically comprising monocytes, eosinophils, and basophils), GRAN#: Absolute Granulocyte count, LYMPH%: Percentage of Lymphocytes among total WBCs, MID%: Percentage of Mid cells among total WBCs, GRAN%: Percentage of Granulocytes among total WBCs

Table 5: Effect of 14-Days Oral Administration of Methanol Leaf Extract of *Vitellaria Paradoxa* on Red Blood Cell Indices in Wistar Rats

Parameters (Unit)	Normal Saline	VPME 250mg/kg	VPME 500mg/kg	VPME 1000mg/kg	P-Value
RBC ($\times 10^6/\mu L$)	5.73 ± 0.31[a]	5.90 ± 0.72[a]	5.97 ± 0.15[a]	5.87 ± 0.71[a]	0.957
HGB (g/dL)	12.03 ± 0.90[a]	11.30 ± 2.66[a]	12.43 ± 1.40[a]	10.37 ± 1.64[a]	0.533
HCT (%)	34.67 ± 3.51[a]	33.00 ± 8.72[a]	35.00 ± 4.00[a]	30.33 ± 7.02[a]	0.787
MCV (fL)	85.00 ± 5.67[a]	84.63 ± 4.79[a]	87.77 ± 2.47[a]	84.00 ± 4.35[a]	0.747
MCH (pg)	32.97 ± 4.88[a]	32.23 ± 5.61[a]	29.43 ± 1.24[a]	32.93 ± 4.91[a]	0.747
MCHC (g/dL)	33.20 ± 0.52[a]	33.70 ± 0.72[a]	33.53 ± 0.95[a]	33.37 ± 0.23[a]	0.812
RDW-CV (%)	15.73 ± 3.32[a]	19.47 ± 3.15[a]	17.57 ± 5.60[a]	15.73 ± 3.32[a]	0.631
RDW-SD (fL)	39.67 ± 2.14[a]	40.43 ± 0.81[a]	39.20 ± 1.87[a]	39.67 ± 2.14[a]	0.869

Values are Means ± Standard Deviation. Mean (s) with the same super script in each row are not significantly different at 5% level of significance (P>0.05). RBC = Red Blood Cell Count, HGB= Hemoglobin concentration, HCT= Hematocrit, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW = Red Cell Distribution Width – Coefficient of Variation, RDW-SD = Red Cell Distribution Width – Standard Deviation.

Table 6: Effect of 14-Days Oral Administration of Methanol Leaf Extract of *Vitellaria Paradoxa* on Platelet Indices in Wister Rats

Parameters (Unit)	Normal Saline	VPME 250mg/kg	VPME 500mg/kg	VPME 1000mg/kg	P-Value
PLT (×10³/µL)	177.67 ± 22.03	193.70 ± 25.11	177.00 ± 21.07	187.70 ± 26.06	0.79
MPV (fL)	7.67 ± 0.74	8.00 ± 0.56	8.03 ± 0.81	7.53 ± 0.51	0.75
PDW (fL)	16.47 ± 8.86	17.63 ± 9.11	10.50 ± 4.68	17.70 ± 9.00	0.67
PCT (%)	0.16 ± 0.05	0.21 ± 0.07	0.15 ± 0.05	0.20 ± 0.10	0.67
P-LCC (×10⁹/L)	38.67 ± 4.62	37.00 ± 1.73	39.67 ± 4.04	38.67 ± 4.62	0.87
PLCR (%)	35.33 ± 5.41	37.07 ± 2.42	33.83 ± 4.52	35.33 ± 5.41	0.86

Values are Means ± Standard Deviation. Mean (s) with the same superscript in each row are not significantly different at 5% level of significance (P>0.05). PLT = Platelet Count, MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT (%) = Plateletcrit, P-LCC = Platelet-Large Cell Count, PLCR (%): Platelet-to-Large Cell Ratio

Effects of Methanol Leaf Extract of Vitellaria Paradoxa on the Histopathology of Kidney of Wistar Rats

The methanol leaf extract of *Vitellaria paradoxa* showed lymphocyte hypertrophy (LH) at 250 mg/kg dose, lymphocyte hypertrophy (LH), slight or moderate tubular necrosis (TN) and tubular atrophy (TA) at 500 mg/kg and 1000 mg/kg doses compared to the negative control group (normal saline) which showed normal glomeruli (G) and normal tubules (T) (Figure 1).

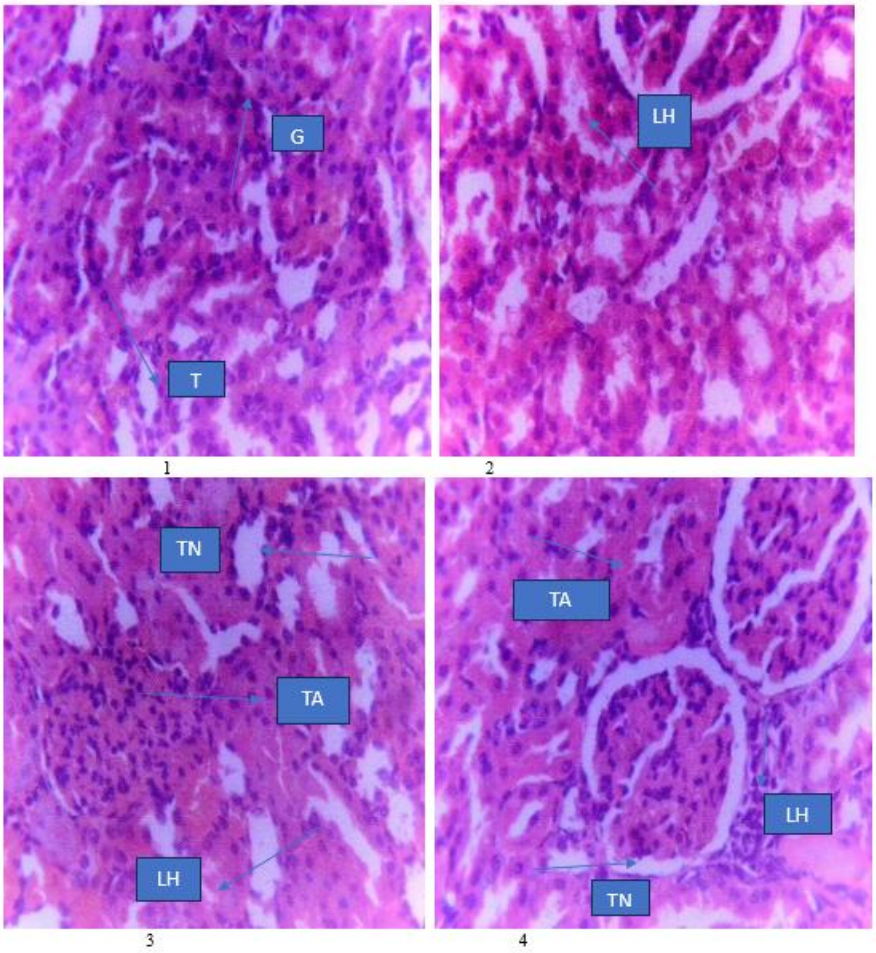

Figure 1: Photomicrographs of the Kidneys of Rats Administered with 250, 500 and 1000 mg/kg of the Methanol leaf Extract of *Vitellaria paradoxa* Compared with Control (Normal Saline) H and E stain (x 400 Magnification)

1= Control (normal saline) showing normal glomeruli (G) and tubules (T)
2= 250 mg/kg extract showing lymphocyte hypertrophy (LH)
3= 500 mg/kg extract showing slight tubular necrosis (TN), tubular atrophy (TA) and lymphocyte hypertrophy (LH)
4= 1000 mg/kg extract showing slight tubular necrosis (TN), tubular atrophy (TA) and moderate lymphocyte hypertrophy (LH)

Effect of Methanol Leaf Extract of Vitellaria Paradoxa on Histopathology of Liver of Wistar Rats

The methanol leaf extract of *Vitellaria paradoxa* showed slight hepatic necrosis with vacuolation and pyknosis at 250 mg/kg dose of the extract. Slight hepatic necrosis (HN) was also observed in the 500 mg/kg and 1000 mg/kg doses of the extract. The control group (normal saline) showed normal hepatocytes (Figure 2).

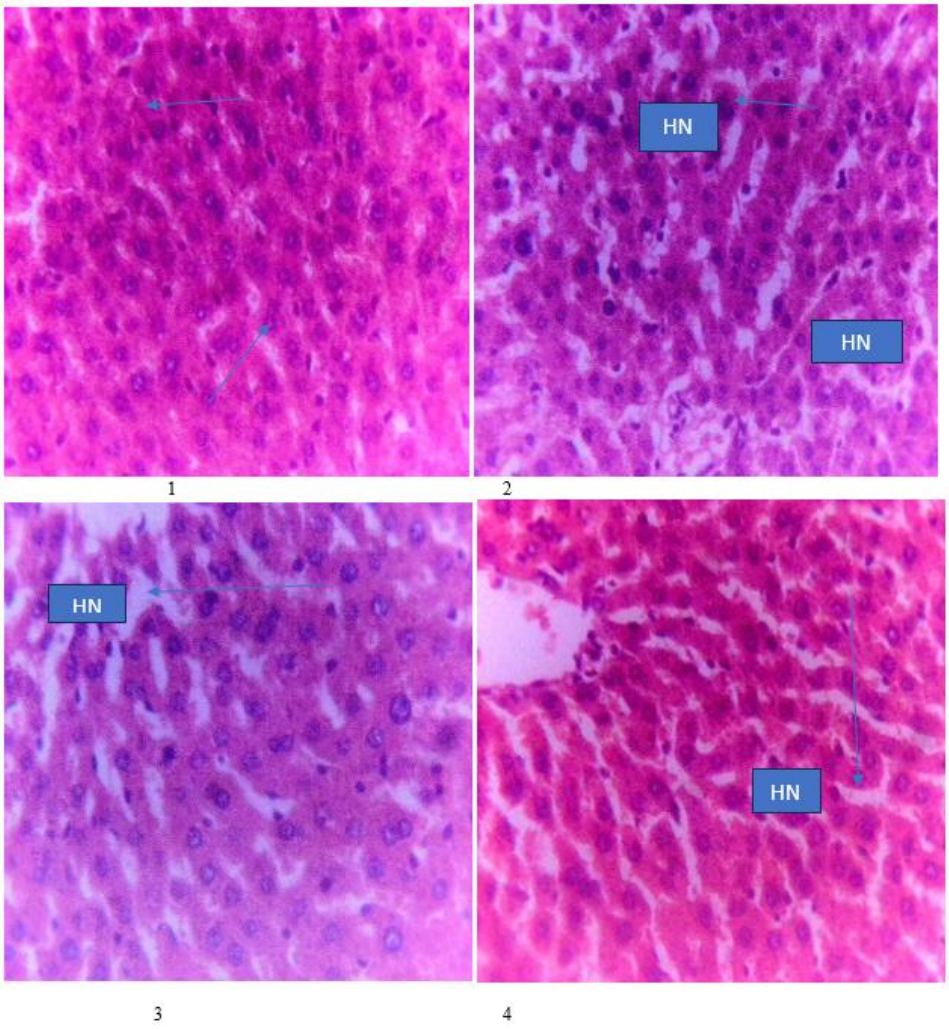

Figure 2: Photomicrographs of the Liver of Rats Administered with 250, 500 and 1000 mg/kg of the Methanol Leaf Extract of Vitellaria Paradoxa Compared with Control (Normal Saline) H and E Stain (x 400 Magnification)

1= Control (normal saline) showing normal hepatocytes
2= 250 mg/kg extract showing slight hepatic necrosis (HN) with vacuolation and pyknosis
3= 500 mg/kg extract showing slight hepatic necrosis (HN)
4= 1000 mg/kg extract showing slight hepatic necrosis (HN)

Discussion

Phytochemical screening of the extract revealed the presence of carbohydrates, alkaloids, flavonoids, saponin, tannins, steroids/terpenes and cardiac glycosides. These compounds are known for their antioxidant and anti-inflammatory properties (Karou *et al.,* 2005). However, these bioactive compounds can also exhibit toxic effects at high concentrations or prolonged exposure. Tannins can impair nutrient absorption and cause hepatotoxicity in excessive doses (Chung *et al.,* 1998; Francis *et al.,* 2002) and the slight hepatic necrosis observed in the histological examination of the liver may be due to the presence of this phytochemical in the extract which are known to exert toxicity at high doses (Francis *et al.,* 2002). The oral LD_{50} of the methanol leaf extract of *Vitellaria paradoxa* was estimated to be greater than 5000 mg/kg and there were no observed adverse effects (NOAEL) nor mortality, suggesting that the extract is safe. The result of biochemical studies showed that there were increased levels, even though insignificant, of ALT and AST at 250 and 500 mg/kg doses of the extract which may be an indication of mild hepatocellular stress but these increases became significant at the highest dose of 1000 mg/kg of the extract tested and this result aligns with the slight hepatic necrosis seen in the histopathological studies of the liver. However, ALP levels did not show significant changes, suggesting that the extract did not affect biliary function. Total protein (TP) levels increased significantly at the highest dose ($P = 0.016$), while albumin levels (Alb) remained stable across groups. The rise in TP may be linked to enhanced protein synthesis which could indicate tissue repair or

immune modulation, while stable albumin levels indicate preserved liver synthetic function (Burtis, Ashwood & Bruns, 2017). Results of histological studies showed that the extract produced slight hepatic necrosis with vacuolation and pyknosis even at the lowest dose of 250 mg/kg of the extract which may be due to elevated levels of ALT and AST as seen in the biochemical studies. The presence of vacuoles (clear spaces) within hepatocytes may be as a result of lipid accumulation (steatosis) and stress and pyknosis is also a sign of cell damage. Alkaloids and especially pyrrolizide alkaloids (PAs) induce hepatocyte necrosis that may progress to liver failure (Diaz, 2015). Hydrolyzable tannins or products of their degradation such as pyrogallol are hepatoxic (Reed, 1995) and the tannins and alkaloids in the methanol leaf extract of *Vitellaria paradoxa* may have caused the hepatic necrosis observed on histological examination of the liver of Wistar rats in this research. Measurements of serum levels of urea and creatinine are usually performed to evaluate kidney function (Arsad *et. al*., 2013). Both creatinine and urea levels are elevated in patients with renal malfunction, especially decreased glomerular filtration. In the early stage of kidney damage, increase in serum urea level usually precedes the increase in serum creatinine that is observed in chronic kidney damage (Craig, 2007). Serum creatinine is a significantly more reliable renal function screening test than serum urea because serum urea levels may be affected by dehydration, diet and protein metabolism, which do not affect serum creatinine levels. Although the serum levels of sodium dismutase (SOD) were similar ($p>0.05$) in extract doses of 250 and 500 mg/kg compared to the control, there was a significant decrease ($p<0.05$) in SOD at 1000 mg/kg dose of the extract when compared with the control group and this can lead to oxidative stress in the kidneys. In this study, there were no increases in both urea and creatinine levels in the extract treated groups as compared to the control group (normal saline) which indicates that the kidney function may not have been adversely affected. However, histological studies revealed, lymphocyte infiltration, slight tubular necrosis and tubular atrophy. These effects are mild and may be reversible upon discontinuation of use of the extract. Electrolytes like potassium ions, K^+, chloride ions Cl^-, and bicarbonate ions showed no significant changes, supporting a normal renal homeostasis (Stevens, Coresh, Greene and Levey, 2006). The results of this study revealed no significant changes in white blood cells (WBC) count, lymphocytes (LYMPH), granulocytes (GRAN), or monocytes (MID), indicating no immunosuppressive or inflammatory effect. Similarly, red blood cells (RBC) indices, including haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH), remained stable, suggesting no adverse impact on erythropoiesis or oxygen-carrying capacity (Hoffbrand, Moss, and Pettit, 2016). Platelet parameters, including platelet count (PLT), mean platelet volume (MPV), and platelet distribution width (PDW), showed no significant alterations, indicating maintained hemostatic balance. This result is similar to those of Tanko *et al.* (2013) in their studies of the antibacterial activity and sub-chronic toxicity of *Vitellaria paradoxa* stem bark extract where it was observed that the hematological parameters remained unaltered, except for a significant reduction in platelet count. Oyeleke *et al.* (2015) in their study of the anti-inflammatory and anti-arthritic properties of *Vitellaria paradoxa* methanolic stem bark extract also demonstrated that the extract effectively reduced inflammation without causing significant changes in hematological parameters. This supports the notion that *vitellaria paradoxa* methanol leaf extract possesses therapeutic properties without compromising hematological health

CONCLUSION

The results of this study showed that while the methanol leaf extract of *Vitellaria paradoxa* was non-toxic at a limit test dose of 5000 mg/kg in the acute toxicity test, there were significant increases in serum levels ALT and AST at the highest dose of 1000 mg/kg tested in the sub-acute toxicity test which is consistent with the slight hepatic necrosis and lymphocyte hypertrophy observed in the histological studies. The extract also caused slight tubular necrosis, tubular atrophy and lymphocyte infiltration which are an indication of mild kidney damage which may be reversible on stoppage of administration of the extract. *Vitellaria paradoxa* methanol leaf extract did not significantly affect all the haematological parameters (WBCs, RBCs and Platelets) measured indicating that the extract has no effect on immunity, erythropoiesis and clotting of blood. It can be concluded, therefore, that methanol leaf extract of *Vitellaria paradoxa* is safe in acute and sub-acute administration, but the extract should be used with caution in chronic disease conditions requiring prolonged administration of the extract.

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