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# Effect of *Vitellaria paradoxa* Stem Bark Ingestion on Kidney Functions in Wistar Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author A. S. Mainasara supervised the study and wrote the manuscript. Author TO designed the study and wrote the protocol. Author UM supervised the study. Author A. S. Mshelia carried out analytical and data analyses. Authors AOM and ASA processed the tissue histologically. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** *Vitellaria paradoxa* is used in the treatment of different diseases in several parts of Nigeria without regard for its safety.
**Aim:** This study was aimed at investigating the effect of ingestion of *Vitellaria paradoxa* stem bark extract on the kidney functions in Wistar rats.
**Place and Duration of the Study:** The study was carried out in the Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

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**Methods:** The oral acute toxicity ($LD_{50}$) of the extract was determined in 30 Wistar rats divided into six of five per group. Group 1 was the control and received distilled water. Different doses of 5, 50, 300, 2000, and 5000 mg/kg were administered once to the study groups (2, 3, 4, 5 and 6) respectively. A sub-chronic toxicity study was carried out in 30 Wistar rats, divided into six of 5 rats per group. Group 1 served as control and was given distilled water and standard rat pellets. The remaining 5 groups were administered different doses of 50, 100, 200, 300 and 400 mg/kg of the extract respectively daily for 30 days. Urea (Ur), Creatinine (Cr), Sodium ($Na^+$), Potassium ($K^+$), Chloride ($Cl^-$), and Bicarbonate ($HCO^-_3$) were assayed using standard techniques. Body weights of the rats were taken twice weekly.

**Results:** No mortality or signs of toxicity were recorded in the rats after 24 hours and up to 14 days post-oral treatment, an indication that $LD_{50}$ of the extract is greater than 5000 mg/kg. In sub-chronic toxicity study, the results did not show treatment-related abnormalities in all the parameters. There were no significant differences ($p>0.05$) in the values of the control and test groups with the exception of urea which was significantly lower ($p<0.05$) in groups 3(3.67±0.55), 4 (3.53±0.65), 5(2.90±0.80) and 6(5.0±0.69) than the control group (6.27±.31) but were within the reference range. Weekly body weights of the rats showed no significant differences ($p>0.05$) between the control and the test groups. Histology results revealed normocytic normochromic cells.

**Conclusion:** From our findings, ingestion of *Vitellaria paradoxa* stem bark extract produced no harmful effect on kidney function in Wistar rats.

*Keywords: Kidney function; toxicity; Vitellaria paradoxa; Wistar rats*.

## 1. INTRODUCTION

A medicinal plant is any plant which contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. Traditional medicine is the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses [2].

*Vitellaria paradoxa* or, Shea tree belongs to the Sapotaceae family. It is a popular tree with several applications in folkloric medicine. The tree grows naturally in the wild of the dry savannah belt of West Africa and stretches in abundance onto the foothills of the Ethiopian mountains [3]. Traditionally, *Vitellaria paradoxa* has been employed in the treatment of several ailments. It promotes wound healing and soothes skin irritation, also used to treat inflammation, rashes in children, dermatitis, chapping and ulcers, as well as rub for rheumatism [4]. Its leaf decoctions are used for stomach ache, headache and as an eye lotion. Roots and root bark are grounded to paste and taken orally to cure jaundice, or they are boiled and pounded to treat chronic sores. They are also used for the treatment of gastric problems as well as diarrhoea and dysentery. Bark decoction is used to facilitate childbirth and to encourage lactation after delivery, or as a footbath neutralizes venom of the spitting cobra [5]. Cosmetics, especially those that prevent skin drying and good looking lipsticks use Shea tree. As a result, cosmetic industries market uses these ingredients in soaps, shampoo and skin cream preparations [5]. *Vitellaria paradoxa* has been studied as a potent medicinal plant [6], against bacterial infections [7] and fungal infections [8]. It has also been reported that *Vitellaria paradoxa* stem bark ingestion caused significant reduction in plasma thyroid hormones concentrations in Wistar rats, malondialdehyde (MDA) was also significantly reduced in the rats but no significance difference in the values of total antioxidant status [9].

The kidney performs a number of essential functions in the body. Most important are the clearance of endogenous waste products, control of volume status, maintenance of electrolyte and acid-base balance, and endocrine function. Metabolism and excretion of exogenously administered therapeutic and diagnostic agents as well as environmental exposures are other major functions [10]. Several therapeutic agents have known nephrotoxic potential; classic examples include anti-microbial agents, chemotherapeutic agents, analgesics, and immunosuppressive agents [11–18]. An important and unregulated source of potentially nephrotoxic substances is the alternative / complementary products, which include herbal remedies, natural products, and nutritional

supplements that are widely available at most health food stores [19,20].

In its role as the primary eliminator of exogenous drugs and toxins, the kidney is vulnerable to develop various forms of injury, our present study was therefore designed to examine in what fashion kidney function will be affected by ingestion of *Vitellaria paradoxa* stem bark in Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Identification

The stem bark of *Vitellaria paradoxa* was collected from Kankia local government, Katsina State, Nigeria, in the month of June. The plant was identified and authenticated at the Herbarium unit of Botany Department, Obafemi Awolowo University, Ile Ife, Nigeria by comparing with established Herbarium specimen with voucher number: Benth 13689 reference number which was kept at the Herbarium.

### 2.2 Preparation and Extraction

Fresh samples of the stem bark of *Vitellaria paradoxa* was collected and air-dried at room temperature over a period of 6 weeks. It was then crushed manually using mortar and pestle. Five hundred grams (500 g) of the grinded plant material was soaked in 4 litres of methanol for 72 hours on a mixer to ensure maximum extraction by percolation method using maceration technique under room temperature. This was followed by periodic stirring. The resulting crude extract was filtered using Whatman number 1 filter paper and then the filtrate was concentrated in an oven at 48ºC to obtain 40 g brown powder extract. The dried crude extract was stored in a refrigerator at low temperature (4ºC) in sterile plastic bottles, at the Faculty of Pharmaceutical Sciences, UDUS, until required for use [8].

The residual filtrate was later re-constituted [8], taking into consideration the average weight of the albino rats, the duration of extract administration, and the required volume of doses.

The crude extract was diluted in cold water to obtain varying concentrations of the extract per Kg body weight. It was then kept refrigerated at 4ºC until use.

### 2.3 Experimental Animals

Albino Wistar rats aged 8 to 12 weeks old, weighing between 100 g to 150 g were obtained from the animal house, of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were allowed to acclimatize for a period of 2 weeks. They were maintained in clean metabolic cage-sand, placed in a well-ventilated room conditions with a temperature of 26ºC to 28ºC, photoperiods of 12 hours light and 12 hours darkness; humidity of 40% to 60% [21].

The animals were maintained on pellet feeds (Vital®), obtained from Grand cereals oil mills limited, Jos and were supplied with drinking water ad libitum. Cleaning of the animal cages was carried out daily, and on regular basis. All the experimental protocols were in compliance with our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National Institute of Health [22] and also in accordance with the recommendations of the International Association for the Study of Pain (IASP) [23].

### 2.4 Experimental Design

#### 2.4.1 Acute toxicity study

Acute toxicity study was performed in accordance with the procedures outlined by the Organization for Economic Co-operation and Development guidelines (OECD) 420 [24]. Thirty Wistar albino rats of both sexes were used for this study. The rats were randomly divided into 6 groups comprising of 5 animals each, with the first group as the control. The extract was administered to rats in groups 2 - 6 in single oral doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg and 5000 mg/kg body weight respectively, by intra gastric gavage using oral cannula. The control group (Group 1) received an equal volume of distilled water. Observations of toxic symptoms were made and recorded within the first hour, four hours and subsequently for 24 hours after administration of the extract. Behavioral parameters and mortality were also monitored closely for 14 days. Lethal dose in 50% of the total population ($LD_{50}$) was interpolated using OECD method [24].

#### 2.4.2 Sub-chronic toxicity study

Sub-chronic toxicity study was carried out in accordance with OECD 407 [25] guidelines. Thirty rats of both sexes were divided into six groups of five rats each. Group 1 served as the control and received distilled water as vehicle. Graded doses of the extract were administered

orally to the rats in groups 2, 3, 4, 5 and 6. The doses given to the groups were 50 mg/kg, 100 mg/kg, 200 mg/kg, 300 mg/kg and 400 mg/kg body weight respectively daily for 30 days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality [21].

At the end of the 30 days period, the animals were fasted overnight, and anaesthetized using chloroform anaesthesia. Blood samples were collected from the animals through cardiac puncture, into clean, dry plain containers. The rats were later sacrificed through lumbar dislocation. The blood collected was allowed to clot and then centrifuged at 5000 rpm for 10 minutes. The serum was separated and kept frozen at-4ºC until required for analysis [21].

#### 2.4.3 Body weight

The rats in all the groups were weighed using a sensitive balance, once before commencement of dosing, twice weekly during the period of dosing and once on the day of sacrifice. Doses of the extract administered were adjusted accordingly.

#### 2.4.4 Biochemical analyses

The separated clear sera were used for the assays of Urea [26], Creatinine [26], Chloride, Bicarbonate [27] using Agape reagent kit (Agape Diagnostics, Switzerland GmbH) and, Sodium and Potassium by flame photometry [27].

#### 2.4.5 Histopathological examinations

The rats were sacrificed, the kidney of the rats were carefully removed after sacrificing the animals and transferred into specimen bottles containing 10% formalin for proper fixation. After fixation, the organs were grossed and then processed using automatic tissue processor. This was immediately followed by embedding the grossed tissues in molten paraffin wax. Sections of 5-6µm in thickness were cut and made onto slides. These tissue sections were then stained using Haematoxylin and Eosin method for photo-microscopic examination of general tissue structures [28].

### 2.5 Data Analysis

The data obtained from this study were analyzed using the statistical package for social science (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were represented as the mean ± standard deviation (S.D). Student T-test at 95% confidence interval was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when $p < 0.05$.

## 3. RESULTS

### 3.1 Acute Toxicity Result

Table 1 shows the result of oral acute toxicity in the Wistar rats. The result of oral acute toxicity showed that no death was recorded in the rats after 24 hours and up to 14 days post oral treatment. This indicates that the $LD_{50}$ of the extract was greater than 5000 mg/kg.

### 3.2 Sub-chronic Toxicity

Table 2 shows the results of the effects of intake of *Vitellaria paradoxa* stem bark extract on the kidney of Wistar rats.

Table 3 shows the effects of the intake of *Vitellaria paradoxa* stem bark extract on the mean body weight of Wistar rats.

### 3.3 Histopathological Results

Figs. 1, 2, 3, 4, 5 and 6 represent Groups 1 (Control), 2, 3, 4, 5 and 6 kidney cells (H & E X100). The photomicrographs of kidney tissue sections from control and experimental rats stained with haematoxylin and eosin are shown below. The histopathological examination of all the tissue sections of the control and experimental animals were essentially normal as no pathological lesions were observed.

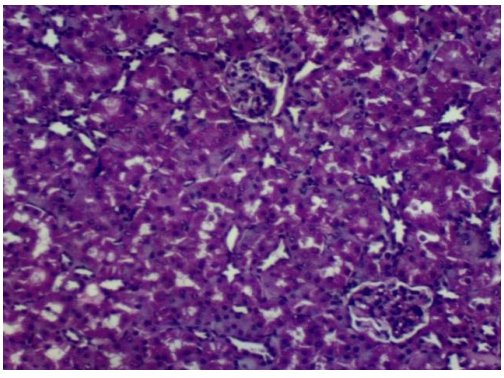

**Fig. 1. Group 1 (Control) kidney cells (H & E X 100)**

*The histopathological examination of the tissue section was essentially normal as no pathological lesion was observed*

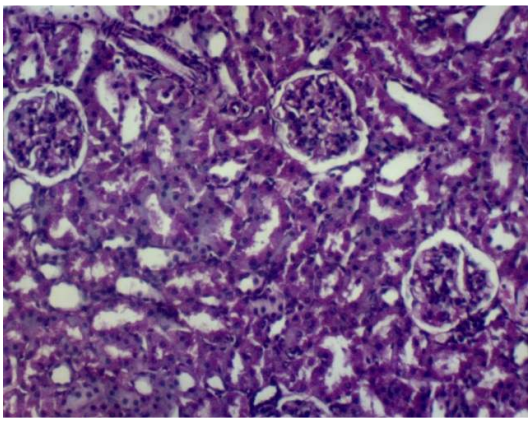

**Fig. 2. Group 2 kidney cells (H & E X 100)**
*The histopathological examination of the tissue section was essentially normal as no pathological lesion was observed*

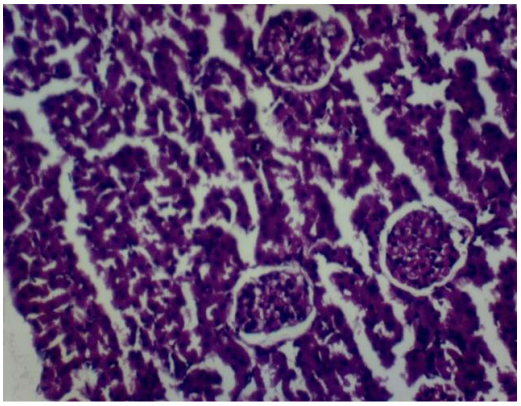

**Fig. 3. Group 3 kidney cells (H & E X 100)**
*The histopathological examination of the tissue section was essentially normal as no pathological lesion was observed*

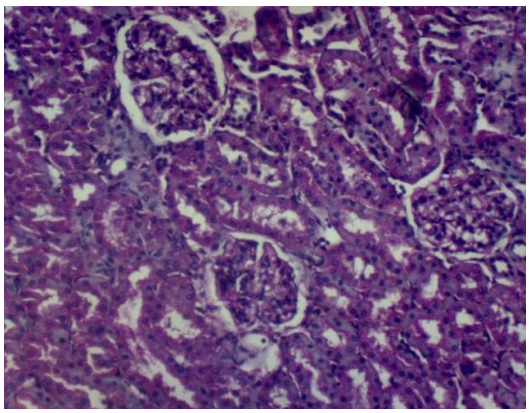

**Fig. 4. Group 4 kidney cells (H & E X 100)**
*The histopathological examination of the tissue section was essentially normal as no pathological lesion was observed*

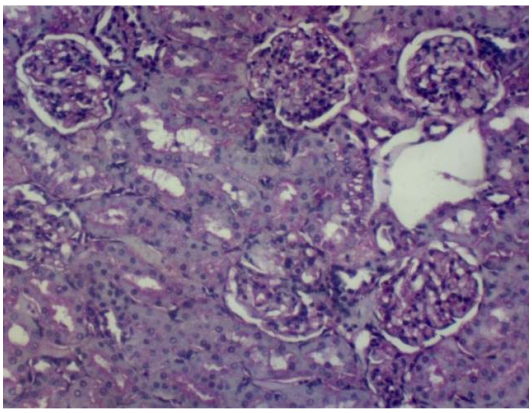

**Fig. 5. Group 5 kidney cells (H & E X 100)**
*The histopathological examination of the tissue section was essentially normal as no pathological lesion was observed*

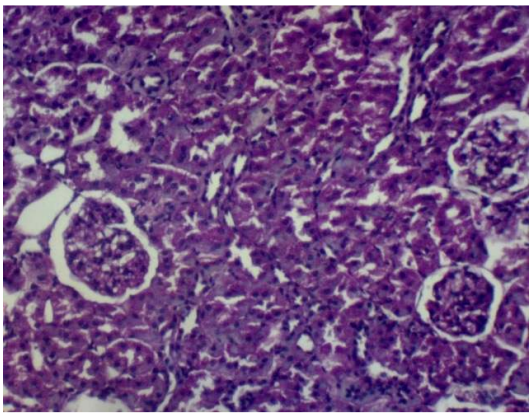

**Fig. 6. Group 6 kidney cells (H & E X 100)**
*The histopathological examination of the tissue section was essentially normal as no pathological lesion was observed*

## 4. DISCUSSION

Drug-induced kidney disease occurs primarily in patients with underlying risk factors. A number of factors enhance the vulnerability of the kidney to the nephrotoxic effects of drugs and toxins. They are broadly categorized as patient-specific, kidney-related, and drug-related factors [10]. The incidence of renal disease is increasing dramatically and has become a significant public health problem both economically and medically [29]. Several studies have indicated the possibility that the use of plant extracts in high doses could lead to toxic injury to the kidneys which interfere with renal tubular functions and induce acute renal failure [28]. From this study, no death or behavioural changes was recorded

**Table 1. Acute oral toxicity ($LD_{50}$) study of *Vitellaria paradoxa* stem bark extract in Wistar rats**

| Groups | No. of animals | Dosage/Kg body weight | Volume of extract (ml) | Observation period | Behavioural changes | Mortality |
|---|---|---|---|---|---|---|
| 1 | 5 | Distilled water | 3 | Up to 48 hours | None | None |
| 2 | 5 | 5 mg/kg | 3 | Up to 48 hours | None | None |
| 3 | 5 | 50 mg/kg | 3 | Up to 48 hours | None | None |
| 4 | 5 | 300 mg/kg | 3 | Up to 48 hours | None | None |
| 5 | 5 | 2000 mg/kg | 3 | Up to 48 hours | None | None |
| 6 | 5 | 5000 mg/kg | 3 | Up to 48 hours | None | None |

**Table 2. Effect of *Vitellaria paradoxa* stem bark extract on the kidney function of Wistar rats**

| Parameters | Group 1 control | *Vitellaria paradoxa* stem bark extract | | | | |
|---|---|---|---|---|---|---|
| | | Group 2 50 mg/kg | Group 3 100mg/kg | Group 4 200 mg/kg | Group 5 300 mg/kg | Group 6 400 mg/kg |
| Urea (mMol/L) | 6.27±0.31 | 6.50±0.56 | 3.67±0.55* | 3.53±0.65* | 2.90±0.80 * | 5.0±0.69* |
| Creatinine (mg/dL) | 0.50±0.10 | 0.57±0.06 | 0.53±0.06 | 0.60±0.00 | 0.50±0.10 | 0 .53±0.06 |
| Sodium (mMol/L) | 155.33±13.61 | 143.33±9.24 | 139±1.73 | 134.33±1.53 | 139±3 .60 | 140±2.00 |
| Potassium (mMol/L) | 4.10±0.26 | 3.60±0.17 | 4.17±0.15 | 5.23±0.96 | 4.20±0.26 | 4 .73±0.25 |
| Chloride (mMol/L) | 104.67±6.80 | 99.67±5.03 | 98.67±2.52 | 99.00±1.00 | 103±4. 36 | 105±0.00 |
| Bicarbonate (mMol/L) | 27.33±0.58 | 24.33±3.05 | 24.33±1.15 | 23.67±3.06 | 23.33±2 .08 | 22.33±0.58 |

** p<0.05= Statistically significant; Number of animal per group = 5*

**Table 3. Mean body weight of Wistar rats after 30 days treatment with *Vitellaria paradoxa* stem bark extract**

| Weeks | Group 1 control | Extract | | | | |
|---|---|---|---|---|---|---|
| | | Group 2 50 mg/kg | Group 3 100 mg/kg | Group 4 200 mg/kg | Group 5 300 mg/kg | Group 6 400 mg/kg |
| Week 1 | 133.83±23.39 | 139.00±7.38 | 127.83±7.49 | 129.33± 1.97 | 123.17±8.68 | 108.33±6.74 |
| Week 2 | 138.00±18.75 | 145.50±7.31 | 132.17±10.67 | 133.83±4.07 | 130.33±6.41 | 117.83±4.26 |
| Week 3 | 148.00±12.92 | 160.33±10.17 | 145.83±14.91 | 146.17±2.40 | 140.50±7.87 | 123.00±4.29 |
| Week 4 | 156.83±15.72 | 173.33±10.97 | 156.50±12.05 | 154.83±7.36 | 152.50±10.63 | 129.00±2.19 |

*Mean body weight ± S.D; * p<0.05= statistically significant; Number of rats per group= 5*

in acute oral toxicity study in all the groups within 24 hours and for up to 14 days, an indication that the oral acute toxicity ($LD_{50}$) of the *V. paradoxa* stem bark is greater than 5000 mg/kg. From the present study, all the parameters assayed revealed no dose related relationship.

There was no significant difference ($p>0.05$) in the values of electrolytes ($Na^+$, $K^+$, $HCO^-_3$, $C^{l-}$) between the control group and the experimental rats. This is an indication that the extract did not induce electrolytes imbalance in the rats. There was also no significant difference ($p>0.05$) in the creatinine values between the control and the experimental rats. The urea values of groups 3, 4, 5 and 6 were significantly lower than the control groups but the values were within reference range [30]. The reduction did not follow a definite pattern as the values fluctuated within the reference range hence there was no dose-related relationship. Besides, all the known causes of low urea values, like low body muscle mass, low protein intake, liver failure etc. were absent in the rats throughout the experiment. It has also been documented that *V. paradoxa* stem bark ingestion have no hepatotoxicity effect in Wistar rats [31]. There was no significant difference ($p>0.05$) in the weights of both groups of the rats. Normal plasma level of urea and creatinine is an indicator of normal kidney function. The kidney histology revealed normochromic normocytic cells.

## 5. CONCLUSION

In conclusion, the present findings, however, have shown that stem bark extract of *Vitellaria paradoxa* was relatively safe and may not produce toxic effects on kidney.

## CONSENT

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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