Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 10 (2)

The Effect of Methanolic Extract of *Vitellaria Paradoxum* Fruit (Shea Nuts) on the Kidneys of Adult Wistar Rats.

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The effects of methanolic extract of pulverized *Vitelleria paradoxum* on the kidney were investigated. The plant is known for its exotic medicinal use; as a source of oil and herbal formulations for treating skin diseases and ailments such as sprains and diarrhoea in West Africa. Twenty-Eight (28) adult Wistar rats weighing between 195g and 215g were used for the study. The animals were randomly divided into four (4) groups, namely: A, B, C, and D. They were respectively administered daily 2.0mls distilled water; 50mg./Kg. body weight; 100mg/kg body weight and 200mg/kg body weight of the extract, with the aid of an oral cannula, throughout the four weeks duration of the experiment. All animals received humane care in accordance with the Guidelines of the Faculty of Basic Medical Sciences, University of Ilorin. All the Wistar rats were given water *ad libitum*. They were all also fed with growers' marsh feeds. At the end of the administration, all the animals were sacrificed by cervical dislocation. Blood samples were taken from the right ventricle into heparinized tubes for haematological assessment as well as plain containers for serum urea analyses. The kidneys were harvested and fixed by immersion in 10% formosaline. Routine tissue processing procedures and staining with Haematoxylin and Eosin (H&E) stains were done for light microscopy. Histopathological studies following oral administration of the extract on the kidney revealed acute tubular necrosis, lobulation and wide urinary space due to shrunken glomeruli with increasing dosages above 50mg/Kg body weight. Haematological profile, however, showed no significant changes in blood cell parameters except for the platelets which were statistically significantly reduced compared to those of the control group ($p < 0.05$). Urea levels were also increased in the plasma suggestive of compromise in the excretory capability of the kidney tubules. Long-term intake of *Vitellaria* paradoxumkernels at high dose is deleterious to the kidney in adult wistarrats and may pose a treat of renal failure in the circumstance of its prolonged endogenous consumption as herbal drug.

Key words: Methanolic extract; *Vitellaria paradoxum* fruit (Shea nuts); kidney; Adult Wistar rats.

INTRODUCTION

The shea butter tree, *Vitellaria paradoxum*Gaertner, belongs to a family called *sapotaceae*. It is particularly found in savanna areas, mostly common in towns, villages, farmlands and forests. *Vitellaria paradoxum* plant has distinguishing features such as long leaf stalks, with more spaced nerves, abundant white latex when slashed and in the petiole of its leaves "shea butter".

Shea fruit resembles a small avocado with delicious and flavored pulp when ripe. This pulp is eaten by people when fruit is slightly overripe and falls down from trees generally from the beginning of June to the end of August[1,2]. Pearson in 1976, found that it contains ascorbic acid (196mg /100g), iron (2mg /100g) and calcium (36.4mg /100g)[3].

The kernel of *Vitellaria paradoxum* is a source of a fatty extract which is becoming more popular by the day as a component of cosmetic formulation[4]. This is because it has, for a long timebeen used as a sedative or anodyne in the treatment of sprains, dislocation and in the relief of minor aches and pains. It has also been shown to promote rapid healing of wounds from hot water burns and fire burns and eczema; hence, it is called skin rejuvenator because of its ability to increase melanin secretion[5-8]. The fruit (kernels) of *Vitelleria paradoxum* is round or oval in shape, containing one seed and occasionally two or three seeds at maturity. The fruit changes from green to yellowish-green after becoming soft and edible to hard brown kernels when dry[9]. It is the main source of edible oil for various parts of African countries. It is also a major source of fatty acid and glycerol.

Phytochemical analysis of various parts of *Vitellaria paradoxa* tree has been done[10,11]. Analysis of its kernel revealed the presence of phenolic compounds such as gallic acid, catechin, epicatechin, epicatechin gallate, gallo-catechin, epigallo-cathechin, epicatechin gallate, quercetin and trans-cinnamic acid[2,11]. It has been shown

to be effective bio mass for dye uptake. Many research works have been done on the uses of *Vitellaria paradoxa* kernels; exotic uses, as mentioned above, ranging from anti inflammatory to its healing potentials but none has shown its endogenous effects in the body's biological systems, hence the need for this research work on its effect on the kidney being one of the five - star organs of the body asides from the brain and the heart.

MATERIALS AND METHODS

Animals and Treatment

A total of twenty-eight (28) adult Wistar rats weighing between 195g and 215g were obtained from the Animal Holdings of the Biochemistry Department of the University of Ilorin and brought into the Animal Holdings of the Faculty of Basic Medical Sciences of the same

Institution for this study. The animals were randomly divided into four (4) groups namely.

Group A: control group which received 2.0mls of distilled water throughout the period of the experiment.

Group B: Experimental group which received 50 mg. / kg. body weight extract

Group C: Experimental group which received 100 mg. / kg. body weight of extract

Group D: Experimental group which received 200 mg. / kg. body weight of extract. (Figure I below)

All animals received humane care in accordance with the Guidelines of the Faculty of Basic Medical Sciences, University of Ilorin. All the Wistar rats were given *ad libitum*. They were all also fed with growers' marsh feeds.

Group A, which was the control group, was administered each with 2 mls. per day of distilled water; while the experimental groups B, C and D received respectively 50 mg., 100 mg. and 200 mg. / kg. body weight of the extract daily, with the aid of an oral cannula, throughout the four weeks duration of the experiment.

Table 1: Table showing Experimental Design Protocol for Wistar Rats (details of animal grouping and treatment in each group)

Group	Number of Rats (n)	Daily treatment	Route of Admin.	Duration (Days)
Group A (Control)	7	2.5 mls. Distilled Water	Orogastric	28
Group B	7	50 mg. / $Kg.^{-1}$ body weight of the extract soln.	Orogastric	28
Group C	7	100 mg. / $Kg.^{-1}$ body weight of the extract soln.	Orogastric	28
Group D	7	200 mg. / $Kg.^{-1}$ body weight of the extract soln.	Orogastric	28

Preparation of Extract: The kernels of *Vitellaria paradoxa* (shea nuts) in dried form,which were further sun-dried for 15 days (brownish in colour), were crushed and grinded using a milling machine. The shells were pounded in a mortal and further grinded to obtain a pulverized form and stored in air-tight containers

200 gm. of pulverized *Vitellaria paradoxa* seeds were then dissolved in 2 litres of methanol in a closed jar for 72 hrs., thus allowing for effective extraction of the bioactive components of the seeds. This mixture was then filtered using a sieve and further filtered using Whatmann blotting papers.

The extracting medium (methanol) was then evaporated using a fractional separating column.

A dark brown jelly-like residue was obtained as the extract.

Haematological assessment and evaluation of serum urea levels.

At the end of the administration, all the animals were sacrificed by cervical dislocation, dissected to get blood samples from the right ventricle into heparinized tubes for haematological assessment as well as plain containers for serum urea analyses.

The kidneys for histopathological examination were fixed in 10% formosaline. Following routine tissue processing procedures for light microscopy, the kidneys were embedded with paraffin, sectioned (5 μm. thick) and stained with Haematoxylin and Eosin (H & E) stains.

Figure 2: Table showing the haematological parameters for Wistar Rats (details of animal grouping and treatment in the group)

Table 2: Table showing the haematological parameters for Wistar Rats (details of animal grouping and treatment in the group)

Blood Parameters	Group A (Distilled Water)	Group B (50 mg. / Kg. b.w.)	Group C (100 mg. / Kg. b.w.)	Group D (200 mg. / Kg. b.w.)
Hb (g)%	8.93	13.7	13.05	10.525
PCV	30.5	41.5	38.25	32.50
RBC	5.20	7.30	6.52	5.50
MCH	19.75	18.7	19.0	18.75
MCHC	34.00	33.25	29.75	32.25
WBC	19.75	9.20	7.95	9.87
Platelets	806.50	746.20	684.75	587.75

Analysis of the kernel revealed the presence of phenolic compounds such as gallic acid, catechin, epicatechin, epicatechin gallate, gallocatechin, epigallocathechin as well as quercetin and transcinnamic acid[11,12].

Histological Results: Photomicrographs of sections were taken and the results are as follows.

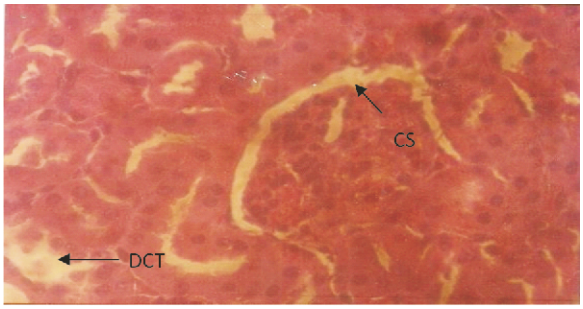

Figure 3: Photomicrograph of the kidney of rat in the Control groups [A], following daily oral administration of 2.5 mls of distilled water for 4weeks showing normal kidney histoarchitecture. [H & E Stain; Mag. X 1200]. Key: CS = Capsular Space and DCT = Distal Convoluted Tubule.

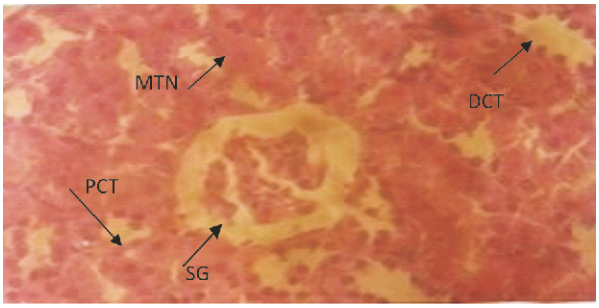

Figure 4: Photomicrograph of the rat's kidney in the Experimental group [B] treated with 50 mg. / Kg. body weight of *Vitellaria paradoxa* for 4weeks showing Shrunken glumerulus (SG), Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT) and Mild Tubular Necrosis (MTN) [H & E Stain; Mag. X 1200].

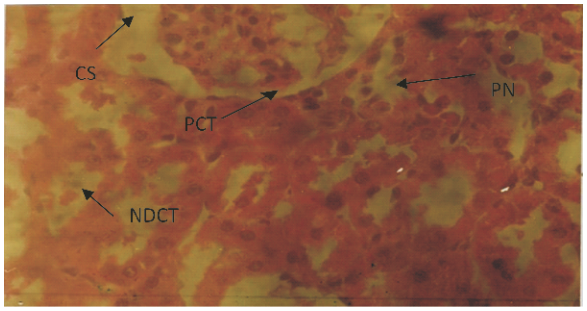

Figure 5: Photomicrograph of the rat's kidney in the Experimental group [C] treated with
100 mg. / Kg. body weight of *Vitellaria paradoxa* for 4weeks showing CS = Capsular Space, PCT = Proximal Convoluted Tubule, NDCT = Necrosed Distal Convoluted Tubule [H & E Stain; Mag. X 1200].

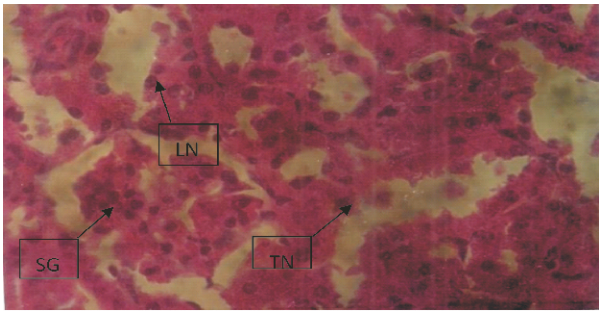

Figure 6: Photo micrograph of the rat's kidney in the Experimental group [D] treated with
200 mg. / Kg. body weight of *Vitellaria paradoxa* for 4weeks showing Shrunken glomerulus (SG); Loss of Nuclei in the tubular parenchyma (LN) and Chronic Tubular Necrosis and distorted tubular parenchyma (TN) [H & E Stain; Mag. X 1200].

The effect of methanolic extract of *Vitellaria paradoxa* on the renal cortex of the kidney of the experimental group with a daily dose of 50 mg. / Kg. body weight, showed light staining nuclei with wide urinary space and shrunken glomerulus (glomerular atrophy).

Histological examination of the slides of rat's kidney in group B (50 mg. / Kg. body weight) as well as Group C (100 mg. / Kg. body weight) of the experimental groups showed no marked changes when examined by a light microscope compared to the control. Group D however revealed marked tubular and glomerular distortion. There was a remarkable loss of nuclei compared to the control as well as of the group with lower dose of the extract if when observedusing a binocular light microscope and magnifications of x 200 and x 800 .Haematological assessment showed no significant change in blood profile at low and mild dose but a significant decrease at higher dosages compared with the control.

DISCUSSION

Herbal drugs are gaining popularity by the day with claims of little or no side effects while been efficacious in the treatment of some diseases by providers of herbal drugs as an alternative therapy. Although *Vitellaria paradoxum* is used more topically and sometimes its fruits are eaten raw, its effect on the kidney and at what concentration it is considered safe has not been ascertained. Although the haematological profile of the experimental group from statistical analysis did not show any significant differences compared to the control, the kidney cells are greatly at risk at increased concentration above 50mg/kg body weight.

Kidneys of Wistar rats to which methanolic extract of *Vitellaria paradoxa* was administered at concentrations of 100 and 200 mg. / Kg. body weight showed histopathological changes in the form of degeneration. The cytotoxic changes observed might be due to dose-dependent toxic effects of the extract as well as its non-nutritional constituents. Lobulation as well as wider urinary spaces characterized the experimental groups with a dosage of 100 mg. and 200 mg. / Kg. body weight.

Mortality and behavioural changes are considered as basic parameters to assess the toxicity of any herbal product, extracts of *Vitelleria paradoxa* kernels has been shown to maintain haematological parameters except for the platelets.

Histopathological effects: Vacuolation was also noticed at a higher dose of 200 mg. / Kg. body weight. This was not observed in lower dose of 50mg/kg and in the control. Nuclei of some deteriorated cells displayed signs of karyorrhesis while a few of the nuclei showed marked karyolysis and changes in parenchyma. Acute tubular necrosis also observed in the groups with higher dose showed diffused interstitial and glomerular haemorrhages which is suggestive of cellular injury affecting both tubular parenchyma and endothelial cells. This is in line with the work of Sujinin 2009[13], which revealed that many herbal formulations showed renal tubular necrosis with extensive interstitial fibrosis and severe tubular loss in the cortex.

Serum urea levels in the experimental groups increased significantly with higher concentrations of 100 mg. / Kg. body weight and 200 mg. / Kg. body weight. The kidney is an essential organ of excretion in the body. It removes nitrogenous wastes, which are byproducts of amino acid metabolism (breakdown) from deamination by the liver. The increase in blood levels of ammonia which is an extremely toxic base and its accumulation in the body would be quickly fatal. An increase in serum levels of urea is suggestive of a compromise in excretory functions of the kidney. Toxicity may be determined by the inability of the kidney to filter and reabsorb the bodily needed threshold substances such as electrolytes[14]**.** Some authors have argued that blood urea and creatinine levels are not sensitive enough in detecting a low level of nephrotoxicity for nephrotoxicants, others have defined nephrotoxicity as increased kidney weight coupled with at least a change in serum parameters such as body electrolytes, urea and creatinine[15]**.** Histopathological changes justified this increase in plasma urea levels with increased dosage of the extract.

Since the source of toxin to kidney cells in *Vitellaria paradoxa* kernels has not been specifically ascertained, it is advisable that herbal preparations for treatment that involve internal ailments using *Vitellaria paradoxum* be taken in limited dosage of less than 100 mg.
/ Kg. body weight or the advice of a physician be sought.

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