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Histopathological evaluation of the kidney of wistar rats following sub- acute administration of methanolic leaves / root extracts of *Thaumatocòccus danieli*.

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Plant extracts are used extensively in traditional medical practise based on the belief that they are rich in bioactive principles, natural and relatively safe, cheaper than orthodox treatment and are readily available. The leaf sap of *Thaumatococcus danielli* is used as an antidote for venoms, stings and bites, while, the leaf exract is used as sedative and to treat insanity. However, there is limited data available on the toxicity profile of this plant. Therefore, this study was carried out to investigate the level of safety of methanol leaf/root extracts of *Thaumatococcus danielli* on the kidney morphology of albino rats. A total of one hundred and forty (140) wistar rats of bth sexes, weighing between (180- 220g) were grouped into 7 of 20 rats in each, while group 1 served as normal control for both extract, other groups were treated with different doses of the leaf and root extracts of *Thaumatococcus danielli*. Doses of the leaf extract used were 8.25, 16.5 and 33mg/kgbw, while doses of 6.25, 12.5 and 25mg/kgbw were selected for root extract. The treatment was for a period of 4 weeks by oral intubation. Kidney histopathology revealed mild tubulonephritis in rats treated with 33mg/kgbw of leaf extract in weeks 3 and 4 and presence of necrotic tissue when treated with 12.5mg/kgbw of root extract in week 4. Thus, continuous ingestion of leaf and root extracts of *Thaumatococcus danielli* especially at high dose can be toxic to the kidney in spite of its nutritional and medicinal benefits.

Keywords: Medicinal Plants; Kidney; Toxicity; Histopathology

INTRODUCTION

Plants have been used in nutrition and folkloric practise to treat certain illnesses over decades. Medicinal plants are used to treat illnesses such as diabetes (Emudainohwo *et al*., 2015), hepatic disorder, arthritis, malaria, gastro-intestinal problems, malaria, worm and insomnia. Some medicinal plants contain diverse groups of bioactive compounds which have been known to help in stabilizing and protecting different internal organs in animals, while others have had varying degree of adverse effect on the organs (Ataman and Idu, 2007). Toxicity testing in animals is carried out on therapeutic products including herbals to identify potential hazards.

Thaumatococcus danielli is a tropical plant found in the rain forest of West Africa particularly Nigeria, Ghana and Cote d'iviore It is the source of the natural sweetener, Thaumatin, which is regarded as a sweet protein (Chinedu *et al*., 2014).

In Nigeria, this plant is used among different ethnic groups for wrapping and boiling food to add to its savour and also to preserve and extend the shelf life of the food. With the widespread uses of the leaves of this plant in food packaging, researches have been made in to its sterility, it was reported that *Thaumatococcus danielli* leaves extracts had no significant antimicrobial activity, but has been shown to possesses hypoglycaemic properties, thus, it can be used in the treatment of diabetes mellitus (Emudainohwo *et al.,* 2015). All the plants part is rich in minerals and crude fibre. The fruit is also a good source of Calcium, Magnesium and Phosphorus (Shalom *et al*., 2014). The high fibre content can be helpful in preventing intestinal and digestive disorders such as constipation, flatulent, pile, colon and rectum cancers (Showemimo and Olarewaju, 2004). *Thaumatococcus danielli* has versatile uses in folk medicine and nutrition. The fruit aril contains the sweetener called Thaumatin which is used in food and confectionery industries as taste and flavour enhancer (Ojekale *et al*, 2007). Thaumatin being a sweet protein and not carbohydrate (Arowosoge and Labode, 2006), has been shown to be very ideal for diabetic as it is non-caloric (Lim, 2012). In folk medicine, the leaf sap is used as antidote against venoms, stings and bites. The fruit is used as laxative (Adeyemi *et al*., 2014). The seed is used as an emetic and for pulmonary problems (shalom *et al*., 2014

The liver and kidney are target organs of attack by toxins including those toxic bioactive principles in plants (Adewunmi and Ojewole, 2004) due to their roles in the homeostatic processes in the body. The mammalian system functions optimally under controlled physiological conditions. This involves a stable equilibrium of the body's internal conditions that ensures the elimination of metabolic waste products.
The kidneys are the major structures responsible for the filtration and excretion of metabolic waste products in the body (Guyton et al., 2011). Conversely, when the kidneys are exposed to lethal doses of toxins or drugs, they may loss excretory functions (Bjornsson, 2017)

The administration of herbal preparation without any standard dosage coupled with insufficient scientific data on their safety is now a matter of great concern especially with the prevalence of liver and kidney diseases.

The use of leaf and root sap of *Thaumatococcus danielli* as sedative and for treating insanity (Shalom *et al.,* 2014) informs this research to investigate into the toxicity profile of this plant.

MATERIALS AND METHODS

Plant Materials & Collection

- Fresh leaves and root of *Thaumatococcus danielli* were collected from the bush at Rukpokwu community of Rivers State, identified in Plant Science and Biotechnology Department, University of Port- Harcourt. The root and leaves (samples), were shade-dried at room temperature to a constant weight, ground to powder with the aid of a blender, A packed into polythene bags and stored in a desiccator for subsequent uses.

Sample extraction

Each powdered sample (leaves and root) was macerated in 1 litre of methanol for 72 hours. This was then filtered using a sieve and whatman No1 filter paper. The filtrate was transferred to a rotary evaporator at 40°C -42 °C and further dried in a water bath at 37°C -40°C. The crude extracts were then stored in a refrigerator at 4°C.

Animal treatment

Adult wistar rats of both sexes, weighing between 180- 220g were bought from the animal house of the department of human Physiology, University of Nigeria, Enugu State and acclimatized for two weeks in the animal house of Biochemistry Department, University of Port Harcourt. During acclimatization, the animals were maintained under standard husbandry conditions in accordance with the Institutional Animal Ethics Committee. Animals were fed with rat pellets and water ad libitum. The leaf and root extracts of *Thaumatococcus danielli* were administered based on the experimental design

Preparation of stock solution

The vehicle used for the reconstitution of the extract was 5% of sodium carbonate (Na_2Co_3), it was prepared by dissolving 5g of Na_2Co_3 in 100ml of distilled water. On the basis of solubility of the extract in the 5% Na_2Co_3 solution, 40mg/ml and 50mg/ml were prepared as follows:
40mg/ml:
This was prepared by dissolving 1g (1000mg) of each extracts (roots and leaves) in 25ml of 5% Na_2Co_3.
50mg/ml:
This was prepared by dissolving 1g (1000mg) of each extract in 20ml of 5% Na_2Co_3.

Experimental Design for Sub-Acute Studies

A total of one hundred and forty (140) wistar rats weighing between (180 220g) were grouped into 7 of 20 rats in each, while group 1 served as normal control for both extract. The treatment was for a period of 4 weeks
For The Roots:
Group 1: Normal control treated with 1ml of distilled water daily
Group 2: Treated with 6.25mg/kg/day (Low dose)
Group 3: Treated with 12.5mg/kg/day (Medium dose)
Group 4: Treated with 25mg/kg/day (High dose)

For The Leaves:
Group 2: Treated with 8.25mg/kg/day (Low dose)
Group 3: Treated with 16.5mg/kg/day (Medium dose)
Group 4: Treated with 33mg/kg/day (High dose)
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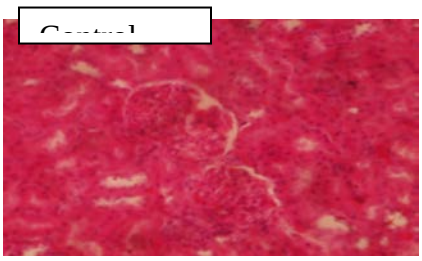
HISTOPATHOLOGICAL EXAMINATION:

The animals were dissected and the liver and kidney collected for histopathological studies. The liver and kidney were fixed in freshly prepared 10% buffered formalin. After 72hrs, the tissues were subjected to automated tissue processing machine with increasing concentration of ethanol. Thin sections of 5um were made using microtome and later stained with hematoxylin and Eosin stain ready for microscopic examination
Histopathological examination of the liver and kidney for inflammation, degeneration and dearrangement was done using the method described by Krause (2001).

Statistical Analysis
Results were analyzed using SPSS (IBM-SPSS). Data obtained were expressed using d+escriptive statistic. Significant difference between the treatment groups and the control was determined using one way analysis of variance (ANOVA). A probability value of less than 5% ($P<0.05$) was considered to be statically significant. Values were expressed in means ± standard error of means

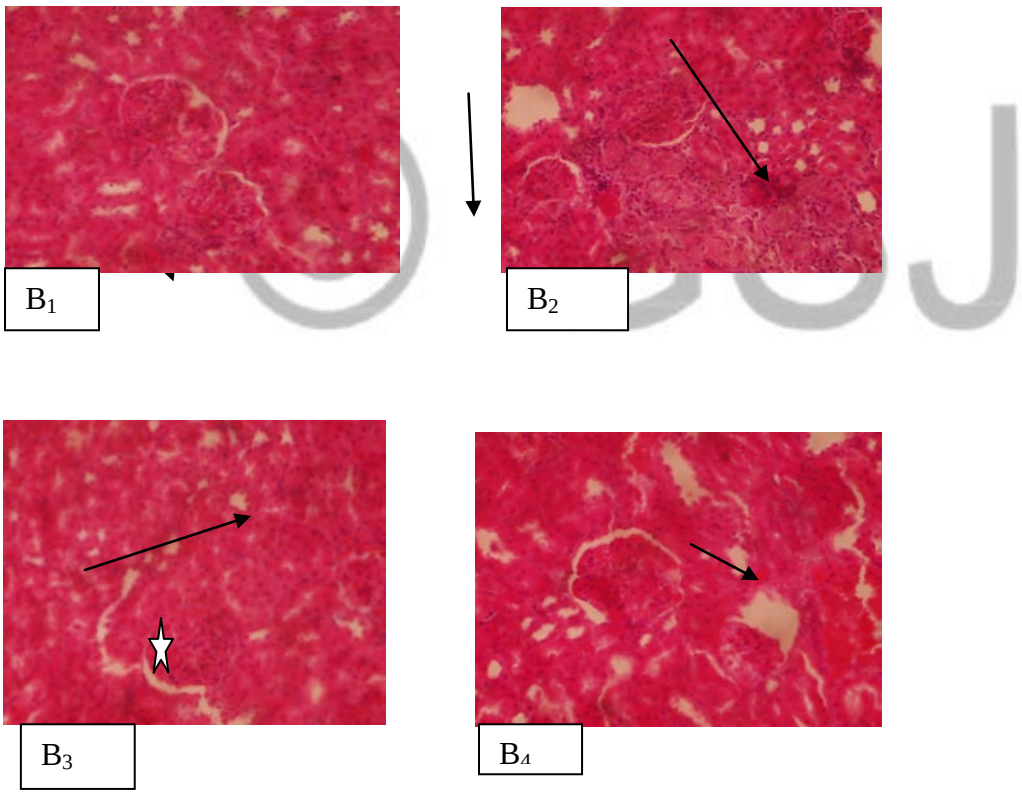
Result

Histopathological Sections of the kidneys



Photomicrograph of rat kidney from the control group. (H&E x400)

A: Photomicrograph of rat kidney from the control group showed normal kidney architecture of the glomeruli, tubules and collecting ducts.



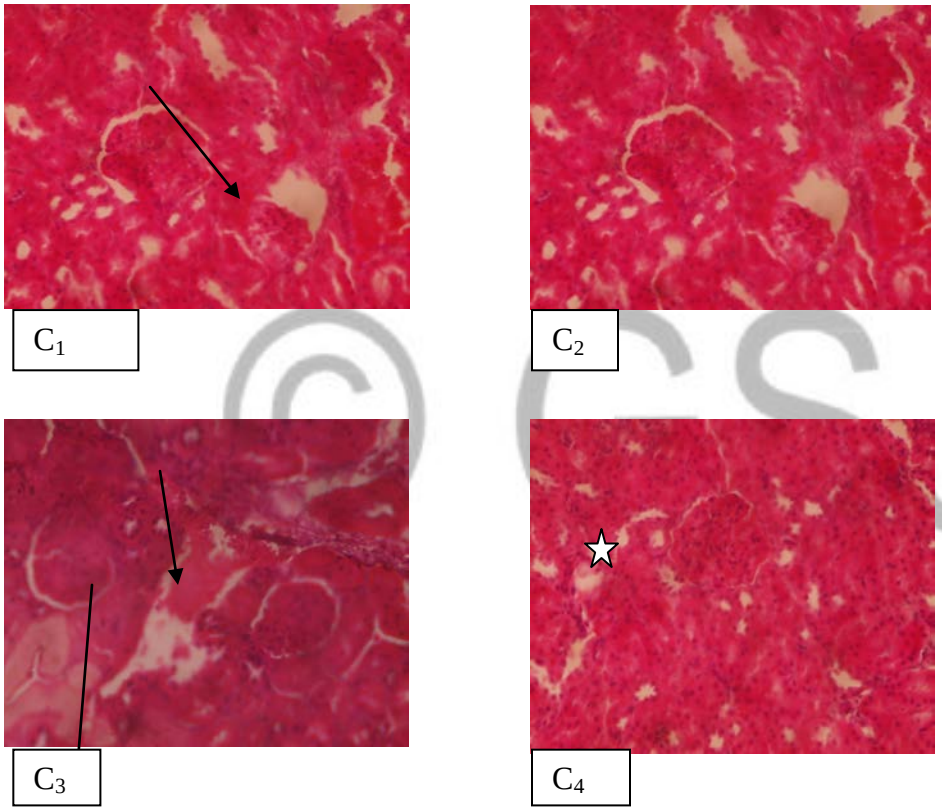
Photomicrograph of kidney tissues treated with 8.25mg/kgbw of methanol leaf extract of *Thaumatococcus danielli* for four weeks.(H&E x400)

PLATE B_1: Photomicrograph of rat kidney at week 1 treatment with low dose (8.25mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showing kidney cortical tissue with some glomeruli indicating normal architecture.

PLATE B_2: Photomicrograph of rat kidney at week 2 treatment with low dose (8.25mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showing kidney tissue with some glomeruli (white star) and tubules (black arrow).

PLATE B_3: Photomicrograph of rat kidney at week 3 treatment with low dose (8.25mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showing normal glomeruli and tubules. The Bowman's capsules are not congested and the tubules do not show any inflammation.

PLATE B_4: Photomicrograph of rat kidney at week 4 treatment with low dose (8.25mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showing glomerular apparatus with mild obliteration of the Bowman's capsule and tubules (white star). There is proliferation of some inflammatory cells (black arrow)



Photomicrograph of kidney tissues treated with 16.5mg/kgbw of methanol leaf extract of *Thaumatococcus danielli* for four weeks. (H&E x400)

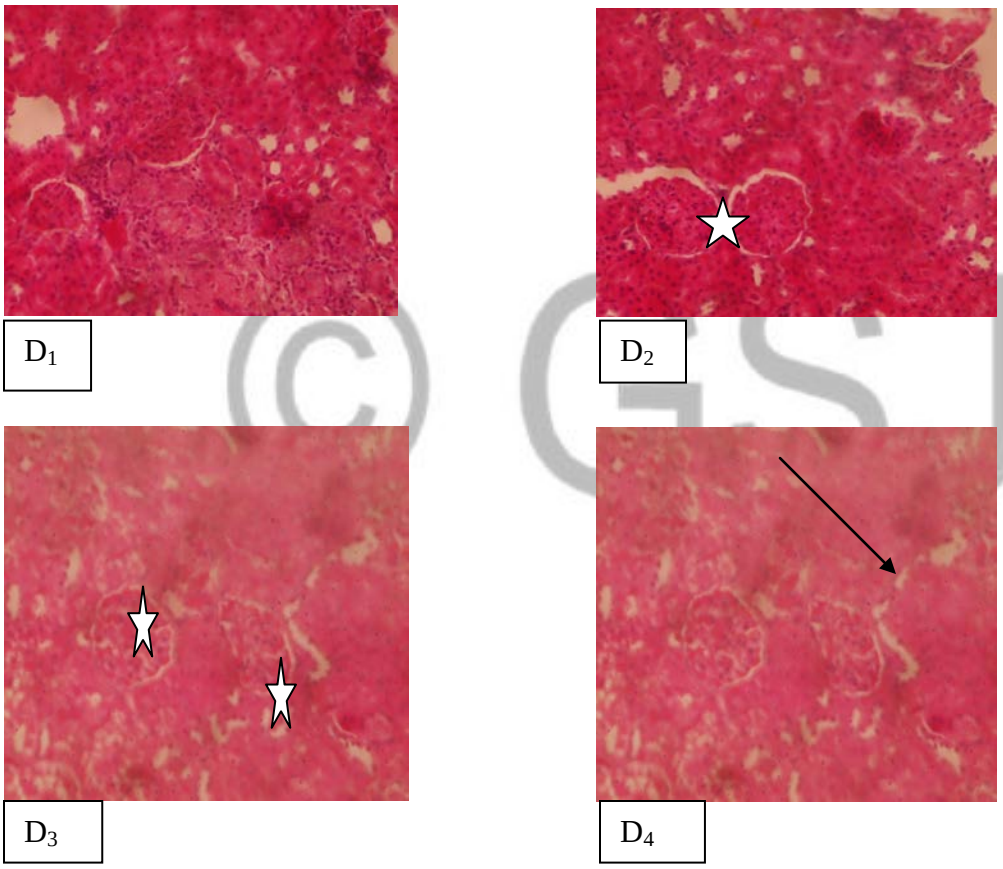
PLATE C_1: Photomicrograph of rat kidney at week 1 treatment with medium dose (16.5mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showing normal glomeruli and tubules (black arrow). The Bowmans capsule is not congested and the tubules do not show any inflammation.

PLATE C_2: Photomicrograph of rat kidney at week 2 treatment with medium dose (16.5mg/kgbw) of methanolic leaf extract of *Thaumatococcus danielli* showing normal glomeruli

and tubules (black arrow). The Bowmans capsule are not congested and the tubules do not show any inflammation

PLATE C_3 Photomicrograph of rat kidney at week 3 treatments with medium dose (16.5mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showed edematous tubules (nephritis) (black arrow).

PLATE C_4: Photomicrograph of rat kidney at week 4 treatments with medium dose (16.5mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showing Kidney cortical tissue with glomerulus (white star). The Bowmans capsular space (black arrow) is not dilated and the tubules do not show any inflammation.



Photomicrograph of kidney tissues treated with 33mg/kgbw of methanol leaf extract of *Thaumatococcus danielli* for four weeks. (H&E x400)

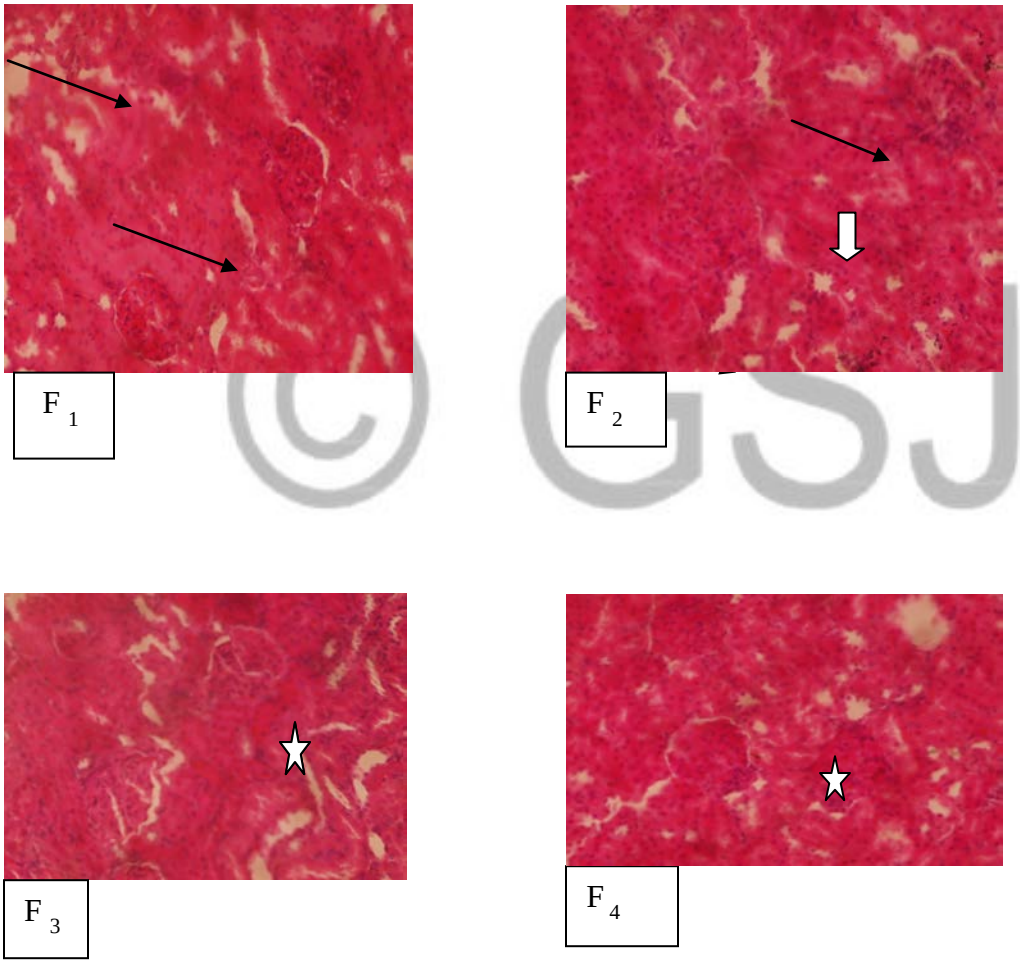
PLATE $D_{1:}$ Photomicrograph of rat kidney at week 1 treatment with high dose (33mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showed well differentiated renal tissue. Picture represents the cortical region of the kidney showing glomerular structures and well defined nucleated tubules. Impression; normal architecture.

PLATE D_2: Photomicrograph of rat kidney at week 2 treatment with high dose (33mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showed some glomerular apparatus (white

star) with mild obliteration of the Bowman's capsule and tubules (white arrow). There are proliferations of some inflammatory cells (black arrow).

PLATE D_3: Photomicrograph of rat kidney at week 3 treatment with high dose (33mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showed some glomeruli (white star). Mild tubulonephritis was observed in the tissue.

PLATE D_4: Photomicrograph of rat kidney at week 4 treatment with high dose (33mg/kgbw) of methanol leaf extract of *Thaumatococcus danielli* showed some glomeruli (black arrow). Mild tubulonephritis was observed in the tissue.



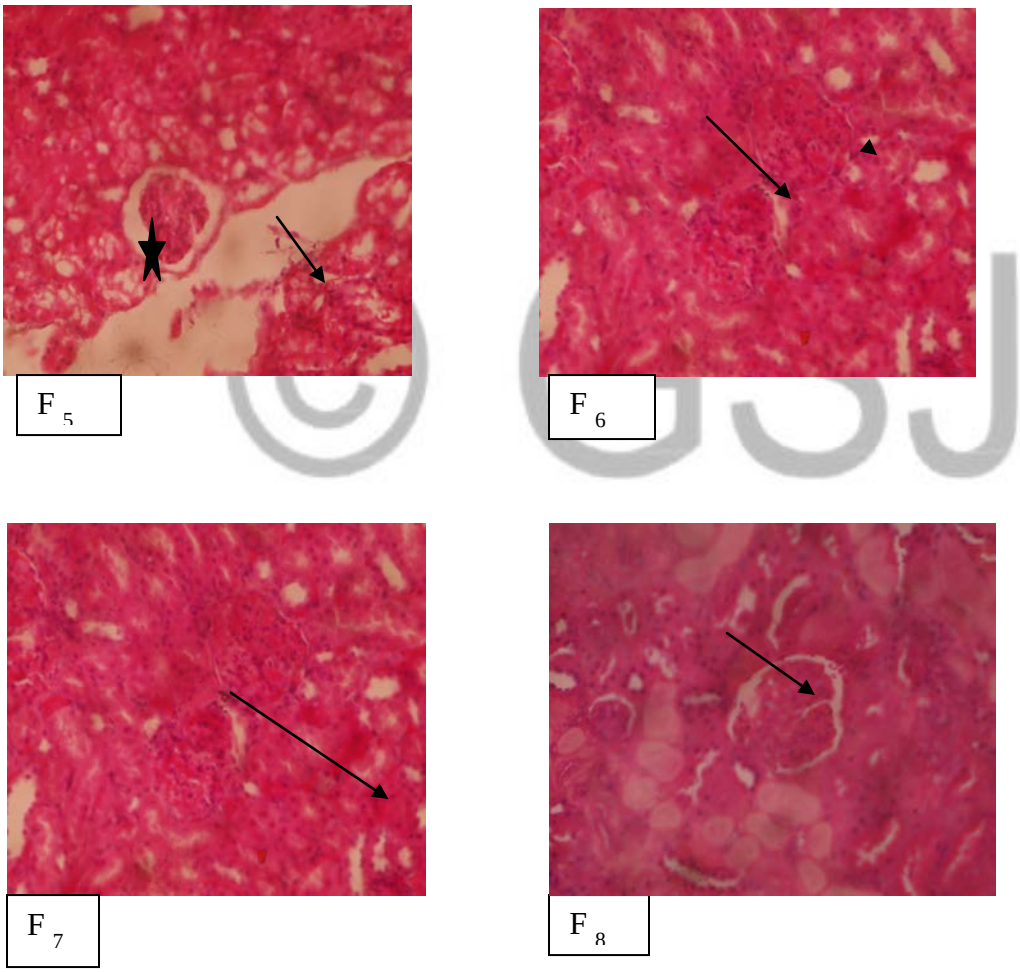
Photomicrograph of kidney tissues treated with 6.25mg/kgbw of methanol root extract of *Thaumatococcus danielli* for four weeks. (H&E x400)

PLATE F_1: Photomicrograph of rat kidney at week 1 treatment with low dose (6.25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed renal cortical tissue with some glomeruli and convoluted tubules. The marked region shows inflammatory congestion of some glomeruli.

PLATE F_2: Photomicrograph of rat kidney at week 2 treatment with low dose (6.25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* revealed kidney glomerulus tissues with a congested bowman's capsule(black arrow) and inflammatory cell proliferation(white arrow).

PLATE F_3: Photomicrograph of rat kidney at week 3 treatment with low dose (6.25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* revealed kidney medullary tissue with few glomeruli and tubules with no depicted abnormality.

PLATE F_4: Photomicrograph of rat kidney at week 4 treatment with low dose (6.25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* revealed kidney glomeruli (white star) with poorly defined Bowman's capsule.



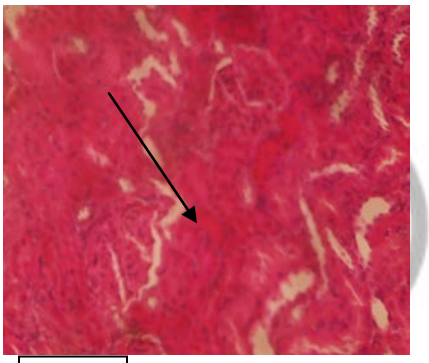
Photomicrograph of kidney tissues treated with 12.5mg/kgbw of methanol root extract of *Thaumatococcus danielli* for four weeks. (H&E x400)

PLATE F_5: Photomicrograph of rat kidney at week 1 treatment with medium dose (12.5mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed glomerulus with dilated Bowman's capsule (black arrow). The tubules are not dilated.

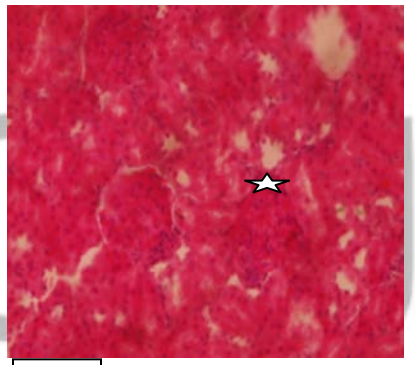
PLATE F_6 Photomicrograph of rat kidney at week 2 treatment with medium dose (12.5mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed kidney tissue with some glomeruli. The Bowman's capsule around these glomeruli was congested (black arrow). The tubules however appeared normal.

PLATE F_7: Photomicrograph of rat kidney at week 3 treatment with medium dose (12.5mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed kidney tissue with some glomeruli. The bowmans capsule around these glomeruli is congested (black arrow). The tubules however appeared normal.

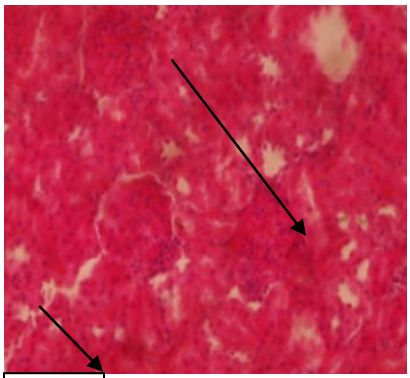
PLATE F_8: Photomicrograph of rat kidney at week 4 treatment with medium dose (12.5mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed inflammed kidney tubules with loss of its cellular architecture. There was also presence of necrotic tissue (black arrow).



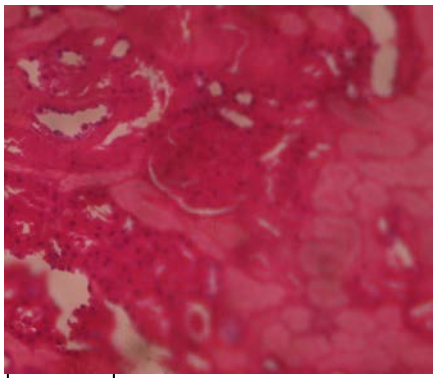
F_9



F_{10}



F_{11}



F_{12}

Photomicrograph of kidney tissues treated with 25mg/kgbw of methanol root extract of *Thaumatococcus danielli* for four weeks. (H&E x400)

PLATE F_9: Photomicrograph of rat kidney at week 1 treatment with high dose (25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed kidney medullary tissue with few glomeruli and tubules. Picture does not depict any abnormality.

PLATE F_{10}: Photomicrograph of rat kidney at week 2 treatment with high dose (25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed glomeruli (white star) with poorly defined Bowman's capsule.

PLATE F_{11}: Photomicrograph of rat kidney at week 3 treatment with high dose (25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed glomeruli with poorly defined Bowman's capsule.
PLATE F_{12} Photomicrograph of rat kidney at week 4 treatment with high dose (25mg/kgbw) of methanol root extract of *Thaumatococcus danielli* showed glomerular with intact Bowman's capsule, but there was loss of pigmentation in the tissue indicating mild edematous degeneration.

Discussion

The adverse effect of drugs including herbal remedies may manifest in form of alterations in the level of biomolecules such as enzymes and metabolic products, normal functioning and histomorphology of the organs (Ashafa *et al*., 2009).

Toxicity studies using animal model helps to assess or extrapolate the potential health risk in humans that may result from toxic components in plant extracts.
The result of kidney histology revealed that the normal control group and low dose (8.25mg/kgbw) group treated with the leaf extract from week 1 to week 3 showed normal histology, but at week 4, mild obliteration of Bowman's capsule and proliferation of some inflammatory cells were observed in the rats. Medium dose (16.5mg/kgbw) treated group showed normal histology, no congestion of bowman's capsule and no sign of inflammation in weeks 1 and 2, but treated rats in week 3 showed some glomeruli edematous tubule (nephritis), while week 4 treatment with 16.5mg/kgbw revealed normal kidney architecture.
High dose (33mg/kgbw) of the leaf extract in week 3 revealed mild tubulonephritis and week 4 treated rats showed normal histology.

It was noted that low dose (6.25mg/kgbw) treatment with root extract in weeks 1 and 2 showed glomerulus with congested Bowman's capsule and proliferation of inflammatory cells, while week 3 treated rats showed no sign of abnormality. Medium dose (12.5mg/kgbw) treatment with root extract in week 1 revealed glomerulus with dilated Bowman's capsule, week 2 and 3 showed normal tubule but congested Bowman's capsule and week 4 treated rats showed inflammed kidney tubule, presence of necrotic tissue and loss of cellular architecture. High dose (25mg/kgbw) treatment with the root extract showed no sign of abnormality in week 1, poorly defined and congested Bowman's capsule in weeks 2 and 3 and loss of pigmentation in the tissue indicating mild edematous degeneration in the fourth week. These observed changes in the kidney tissues such as congested Bowman's capsule, nephritis, presence of necrotic cells and loss of cellular architecture are signs of kidney injury which could have resulted in the

generation of inflammatory mediators (cytokines and chemokines) by innate immune system and infiltration of inflammatory cells (neutrophils, lymphocytes, macrophages and natural killer cells) into the interstitium of the kidney. These pathological changes may be attributed to the phytochemicals (alkaloids, flavonoids, saponins, tannins, phenols, oxalate etc.) contained in the leaf and roots of this plant, suggesting that this plant may be moderately toxic considering the fact that the LD_{50} of the leaf was found to be 330mg/kgbw and that of the root was 250mg/kgbw which falls within the range of moderate toxic drug or substance (Osibemhe *et al*., 2016).

The kidneys are the major structures responsible for the filtration and excretion of metabolic waste products in the body (Guyton et al., 2011). Conversely, when the kidneys are exposed to lethal doses of toxins or drugs, they may loss excretory capacity (Bjornsson, 2017.)

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