

Histomorphological Study of the Effect of Ethanolic Extract of *Nauclea latifolia* on Neonatal Kidney

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Abstract

Histomorphological study of the effect of ethanolic leaf extract of *Nauclea latifolia* on neonatal kidney was investigated. 72 albino wistar rats consisting of 60 females and 12 males weighing between 100-273g were used for this study. This study was divided into 3 phases, each phase consisting of 4 groups (one control group and three experimental groups). LD₅₀ was carried out to determine the doses represented as low dose (500mg/kg), middle dose (1000mg/kg) and high dose (1500mg/kg) of *Nauclea latifolia* leaf extract. In all the 3 phases, the control groups (1A, 2A and 3A) received 10% Tween 80. In phase 1, the experimental animals designated (1B, 1C and 1D) received, 500mg/kg, 1000mg/kg and 1500mg/kg of *Nauclea latifolia* respectively for 21 days before pregnancy. In phase 2, the experimental group animals designated (2B, 2C and 2D) received, 500mg/kg, 1000mg/kg and 1500mg/kg of *Nauclea latifolia* respectively for 21 days before pregnancy and 7th to 13th day of gestation. In phase 3, the experimental group animals designated (3B, 3C and 3D) received 500mg/kg, 1000mg/kg and 1500mg/kg of *Nauclea latifolia* respectively from 7th to 13th day of gestation and the litters were sacrificed within 48 hrs and tissues were processed using haematoxylin and eosin (H & E). The result showed that the extract affects the cytoarchitecture of the neonatal kidney. In phase 1 and the sub-groups, there was an abnormal cellular pattern with area of inflammation in the experimental animals. Phase 2 revealed abnormal cellular patterns with numerous area of necrosis in the entire treated sub - groups while in Phase 3 there was an abnormal cellular pattern with numerous areas of necrosis and vascular degeneration in the experimental animals compared to the control groups. It is evident that *Nauclea latifolia* at low doses, showed mild toxic effect on neonatal kidney and the effect increases tangentially as the doses increased. Gross morphologically, there was significant weight gain in the body weight of the groups compared to the control groups at $p > 0.05$. Thus signifies the use of this plant during pregnancy/gestation impose deleterious effect with histopathological alterations and the usage to be discouraged during pregnancy.

Keywords: kidney, body weight *Nauclea latifolia* and albino wistar rats

1.0 INTRODUCTION

Medicinal plants are re-emerging health aid which was already in use in developing countries. This has become useful because of the rising cost of orthodox drugs in the maintenance of personal health and well being (Delsiva and Hoareau, 2005). The south-eastern inhabitants of Nigeria are known for their high consumption of vegetables (Nwangwu *et al.*, 2009). Some of these vegetables form part of the food consumed on certain health conditions including ill health and in times of convalescence.

This report shows on the role of plant in the life of man from past till date. As an old companion of man, it has provided food, shelter, wealth and has helped to maintain a relatively disease Free State when properly utilized as herbal medicine (Nwangwu *et al.*, 2010). Although modern medicine may be available in developing countries, but the use of herbs for treatment of disease has often maintain popularity for historical and cultural reasons (Nwangwu *et al.*, 2009).

The use of herbs in treatment of disease has gain ground worldwide, making herbal medicine an inevitable global discuss. This practice allowed for research into pharmacological activities of plants secondary metabolites that influence biological process and reverse disease free state (Ugochukwu and Babady, 2003). The herbal plants have also been reported to have fewer side effects (Karim *et al.*, 2011).

Nauclea latifolia belongs to the family of Rubiaceae, the common name include pin cushion tree or African peach. It is locally called "Egbesi" in Yoruba, "Ubuluino" in Igbo, "Marga" in Hausa and "Mbom-ibon" in Ibibio. *Nauclea latifolia* is a small evergreen tree or straggling shrub with leaves rounded ovate, apex shortly acuminate, rounded or lunate base and stipulates ovates (Duke, 2008). Parts used include leaves, roots, stem and fruits. It has been found useful in folk medicine for treatment of malaria, hypertension, diarrhoea, tuberculosis, dysentery and also as a laxative (Okiemy-Andissa *et al.*, 2004). Phytochemical analysis identifies indole-quinolizidine, alkaloids (glycoalkaloids) and saponins as the major components (Karou *et al.*, 2011). Gidado *et*

al., (2005) reported antidiabetic properties for the root and leaf extract while Taiwe *et al.*, (2010) reported antidepressant and anti-anxiety effect for the root extract of the plant. A decoction of the stem in water has been demonstrated to exhibit a high anti-parasitic potential (Benoit-Vical *et al.*, 1998). The aqueous extract of the leaf also showed effectiveness against *Plasmodium falciparum* (Benoit-Vical *et al.*, 1998). Hot aqueous and ethanolic extract was demonstrated to exhibit strong anti-bacterial property (Okiei *et al.*, 2011). Alkaloid rich extract of *Nauclea latifolia* can react *in vitro* with mammalian DNA leading to G₂-M cell cycle arrest and heritable DNA-damage in liver, kidney and blood cells; it induces single-strand breaks (Traore *et al.*, 2011).

Traditionally, *Nauclea latifolia* in West and South Africa, infusions and decoctions of the bark and leaves are used for the treatment of stomach pains, fever and diarrhoea and against parasites, like nematodes in men and animals, and tropical disease like malaria. In Kano (Nigeria) *Nauclea latifolia* is used as chewing stick, as a remedy against stomach ache and tuberculosis (Deeni and Hussain, 1991). In Ivory Coast, infusions and decoctions from stems and roots of *Nauclea latifolia* are used in treatment of malaria by traditional healers (Benoit-Vical *et al.*, 1998).

The leaves of *Nauclea latifolia* have been used in folk medicine for the treatment of malaria, hypertension, diarrhoea, tuberculosis, dysentery and also as laxative (Okiemy-Andisia *et al.*, 2004). The root extract has been reported to have anti-depressant and anti-anxiety effect (Taiwe *et al.*, 2010). The root is also used in the management of diabetes (Gidado *et al.*, 2005). The decoction in water has been demonstrated to exhibit anti-parasitic potential (Benoit-Vical *et al.*, 1998). The aqueous extract of *Nauclea latifolia* is used against chloroquine resistance strains of *Plasmodium falciparum* (Benoit-Vical *et al.*, 1998). The hot aqueous extract and ethanolic extract has been demonstrated to exhibit a high anti-bacterial property (Okiei *et al.*, 2011).

The decoction of the leaves is recommended for stomach upset, especially in children (Gill, 1992). The decoction of the leaves along with alligator pepper is given for cough, cold and general weakness of the body (Gill, 1992). The fruit is recommended for piles, dysentery, colic, emetic and menstrual disorder (Gill, 1992). The root is chewed as chewing stick (Gill, 1992). Other ethnobotanical uses of *Nauclea latifolia* include malaria, leprosy, piles, gonorrhoea, debility, dyspepsia and enteritis (Duke, 2008). The traditional birth attendant in Nigeria have used the ethanolic extract of *Nauclea latifolia* stem and root bark in arresting pre-term contraction in pregnant women (Duke, 2008).

Out of thirty three plants, commonly used in West tropical Africa by traditional healers for treatment of malaria *Nauclea latifolia* showed a good antiplasmodial activity and a weak toxicity. The ethanolic extract obtained by decoction was evaluated *in vitro* against chloroquine-resistant FcB1 strain of *Plasmodium falciparum*. Cytotoxicity was evaluated on the human MRC-5 and the rat line L-6 cell lines (Zirih *et al.*, 2005).



Fig 1. *Nauclea latifolia* leaf (www.google.com)

There is first, preliminary information about the development of suitable tablets dosage form for a medicament against malaria. Studies were done with the water extract of *Nauclea latifolia*. It was oven dried and the mechanical properties were determined. The tablets produced had good mechanical properties like hardness increasing with compression pressure. But the friability decreased and the disintegration was poor. A disintegrating material should be included in the formulation of the tablets (Emeje *et al.*, 2005).

In humans the kidneys are located in the abdominal cavity, more specifically in the paravertebral gutter and lie in a retroperitoneal position at a slightly oblique angle. There are two kidneys. One is on each side of the spine. The asymmetry within the abdominal cavity caused by the liver typically results in the right kidney being slightly lower than the left, and left kidney being located slightly more medial than the right.

The kidney has a bean-shaped structure; each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia (of Gerota) and paranephric fat. The anterior (front) border of these tissues is the peritoneum, while the posterior (rear) border is the transversalis fascia.

The nephron is the functional unit of the kidney and each consist of one renal corpuscle and its associated tubule. The essential tissue composition of kidney is that of a gland with highly modified secretory units and highly specialized ducts. In the kidney, each corpuscle is a highly modified secretory acinus as it secretes a filtrate of blood plasma while each tubule functions as exaggerated striated ducts. Renal tubules have wiggly portions called convulated tubules, straighted segments called loop of henle, and collecting ducts. The kidney accomplishes various homeostatic functions both independently and in concert with other organs such as excretion of waste, e-absorption of vital nutrients, acid-base homeostasis, osmolality regulation, blood pressure regulation and Hormonal secretion

This study was carried out to investigate the effect of *Nauclea latifolia* on the kidney of Neonate owing to the fact the mother, pregnant rats were administered with this extract and the histomorphological effect was evaluated with the view to elucidate the risk of pregnant mothers taken or using the leave extract as one of the remedies during pre-natal or anti-natal care.

Despite the acclaimed and documented uses of *Nauclea latifolia* there appears to be a paucity of information on the safety of this plant on the neonatal kidney, as traditional birth attendant in Nigeria have used the ethanolic extract of *Nauclea latifolia* stem and root bark in arresting pre-term contractions in pregnant women (Duke, 2008).

2.0 MATERIALS AND METHODS

Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonate V, sodium bicarbonate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, distilled water, hutches, concentrate feed, syringe and hypodermic needles, EDTA treated bottles, latex hand glove, weighing scale, graduated vials, measuring tape, they were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

Collection and Preparation of the extract of *Nauclea latifolia*

Fresh leaves of *Nauclea latifolia* was harvested from a local farm in Itu Local Government Area, of Akwalbom State. The samples were clean of dirt and packed in a clean sterile bag and taken to Pharmacognosy Laboratory of the University of Uyo for identification and extraction. The freshly harvested leaves of *Nauclea latifolia* was pulverised using mortar and pestle and extracted with 70% ethanol using soxhlet extractor, and the extract was refrigerated at 4°C until ready for use.

Median Lethal Dose of *Nauclea latifolia*

Lorke's method (LD₅₀) was calculated as geometrical mean of the maximum dose producing 0% mortality (a) and the minimum (LD₅₀= $\sqrt[ab]{ab}$ (Lorke's 1983). The acute toxicity of *Nauclea latifolia* on albino wistar rats was 500, 1000 and 1500mg/kg body weight. All experimental animals were observed for physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, body limb and death within 42hours. The extract was administered intraperitoneally (I.P). 500mg/kg and 20mg/kg per body weight were calculated respectively for *Nauclea latifolia*. Doses were considered as stock solution, they were calculated further using 20mls of distilled water for *Nauclea latifolia*

Experimental Animals

72 adult albino wistar rats consisting of 60 females and 12 males weighing between 100-237g was obtained from the Animal House of the College of Health Science University of Uyo, Uyo Nigeria.

Environmental Conditions

The animals were housed in wooden cages with plated lead net as covering and sawed particles as bedding. The room temperature was between 26°C-29°C and 12:12 light and dark cycles was maintain throughout the period of the experiment. The animals were allowed to acclimatize for 14 days before commencement of the experiment.

Experimental Sites

The study was done at the College of Health Sciences Animal House, University of Uyo, Uyo, Akwa-Ibom State Nigeria.

Experimental Protocols/Design

72 albino wistar rats consisting of 60 female and 12 male weighing 100-237g were used for this study. They were randomly selected into three groups, designated Phase 1, 2 and 3. Each phase consist of 24 rats having a control group and 2 experimental groups.

Administration of extract based on Phases and groups in phases

Control Group: This group consist of control group; the animals were given distilled water conjugated with Tween 80 which served as a dissolving agent for the extract for the experimental groups.

Phase 1: This group consisted of 3 experimental groups; they were given 500mg/kg as Low dose, 1000mg/kg as Middle dose and 1500mg/kg as High dose of ethanolic extracts of *Nauclea latifolia* per body weight of the animal orally for 21days. After 21days the female rats of each group were exposed to the male rats of same group for copulation. When pregnancy was established female rats was withdrawn from males and litters sacrificed within 48hours of delivery.

Phase 2: This group consisted of 3 experimental groups, the female rats of each group were exposed to the male rats of same group for copulation. When pregnancy was established female rats were withdrawn from males and was administered 500mg/kg, 1000mg/kg, and 1500mg/kg of *Nauclea latifolia* leaf extract per body weight of the animals from the 7th day of gestation to the 13th day of gestation and litters were sacrificed within the 48hours of delivery.

Phase 3: This consisted of 3 experimental groups, the female rats were exposed to male rats from the onset, when pregnancy was established, the experimental animals were given oral doses of 500mg/kg, 1000mg/kg and 1500mg/kg of *Nauclea latifolia* extract per body weight of the animal respectively from 7th to 13th day of gestation and litters sacrificed within 48hours of delivery.

Establishment of Pregnancy in the Female Albino rats

After two weeks of acclimatizing the rats, the male and female rats were placed together for copulation, every morning between 8:00am and 10:00am, the weight of the female rats were checked to see if there was any unusual increase in the weight of the animals which may be due to pregnancy. After checking the weight the vaginal smear was collected from the female rats. The vaginal smear taken from the rats was put on frosted glass slides. The slide was viewed under the microscope to check for the presence of sperm cells which confirms pregnancy.

Sacrifice and collection of Organs

At the end of the stipulated administration, 48 hours after delivery, the litters were subjected to a 12 hours fast but had access to water and they were sacrificed using chloroform vapor.

Kidney were carefully harvested out from the litters, harvested organs were carefully dissected out.

Gross morphological analysis

Kidney dissected out were weighed using Digital weighing balance, trimmed of all fat and connective tissue blotted dry to remove any blood and fixed in Bouin's fluid immediately and transported to Research Unit, Gross Anatomy Laboratory for Histopathological analysis.

Statistical Analysis

One way of analysis of variance (ANOVA) was used to compare the groups for means treatment and control, thereafter the post-hoc test using student Newman-keul method was carried out to find the level of significance at $p < 0.05$. All the results were expressed as mean \pm standard error of means.

Histopathological Analysis

After 72 hours after the reception of the organ, kidney in Histopathology laboratory, 0.5-1 mm in thickness were dissected out and post fixed in Neutral Buffered Saline and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas. The sections were designated "vertical sections". Serial sections of 5 μ m in thickness were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

Photomicrography

Records of the Histological and histochemical results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health Sciences, University of Uyo, Uyo, Akwa- Ibom, Nigeria as illustrated in Plate.1 to 12.

3.0 RESULT

Gross morphological findings

PHASE 1

Initial, Final and Weight Gain in the Control and Experimental Rats given extract for 21 Days before Pregnancy.

The result presented in Table 1 revealed a significant different in initial body weight ($F = 27.63, p < 0.05$), final body weight ($F = 35.81, p < 0.05$) and weight gain ($F = 6.84, p < 0.05$) between the control, Low dose, middle dose and high dose group. The mean value of initial body weight in the middle dose group was significantly higher than Low dose and high dose ($p < 0.05$) but insignificantly different from control ($p > 0.05$) and that of the high dose was significantly less than control, low dose and middle dose ($p < 0.05$). the Final weight obtained in middle dose and high dose group were significantly less than control and low dose ($p < 0.05$) while that of the low dose group was significantly less than that of the control ($p < 0.05$). the mean weight gain in the group treated with high dose of the extract was significantly less than control and middle dose ($p < 0.05$) but insignificantly different from low dose ($p > 0.05$).

Table 1

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	140.50 ± 4.50	181.00 ± 8.00	40.50 ± 3.50
Low dose	106.00 ± 6.00	137.50 ± 7.50 ^a	31.50 ± 1.50
Middle dose	180.00 ± 18.00 ^b	227.00 ± 11.00 ^{ab}	47.00 ± 7.00
High dose	229.00 ± 5.00 ^{abc}	242.00 ± 3.00 ^{ab}	13.00 ± 8.00 ^{ac}

Value are reported as means ± SEM, a = significantly different from control ($p < 0.05$), b = significantly different from low dose ($p < 0.05$), c = significantly different from middle dose ($p < 0.05$).

PHASE 2

Initial, Final and Weight Gain in the Control and Experimental Rats given extract for 7 Days after Pregnancy

The result of ANOVA showed an insignificant difference in the mean initial weight ($F = 1.77, p = 0.291, p > 0.05$), and final weight ($F = 0.164, p = 0.915, p > 0.05$) between groups but for weight gain, the result was significantly different ($F = 24.52, P < 0.05$). Those treated with high dose of the extract reported a significantly lesser weight gain relative to control, low dose, middle dose and high dose ($p < 0.05$). Results are as presented in Table 2

Table 2

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	128.50 ± 3.50	181.00 ± 7.00	52.50 ± 3.50
Low dose	146.00 ± 4.00	188.00 ± 5.00	42.00 ± 1.00 ^a
Middle dose	124.50 ± 6.50	176.00 ± 10.00	51.50 ± 3.50
High dose	160.50 ± 23.50	185.50 ± 22.50	25.00 ± 1.00 ^{abc}

Value are reported as means ± SEM, a = significantly different from control ($p < 0.05$), b = significantly different from low dose ($p < 0.05$), c = significantly different from middle dose ($p < 0.05$).

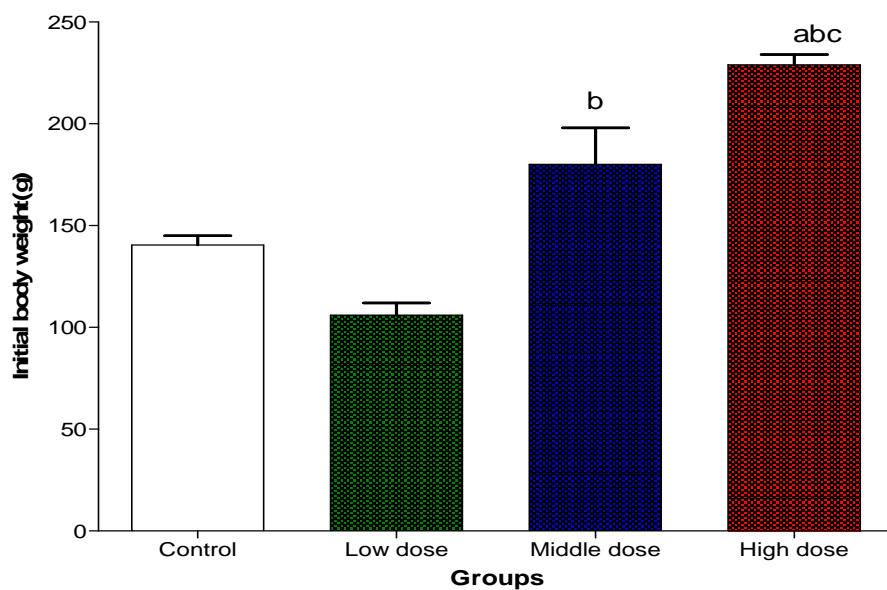


Fig 2: Initial weight Phase 1

Means \pm SEM were plotted, b = significantly different from low dose ($p < 0.05$), abc = significantly different from control, low dose and middle dose ($p < 0.05$).

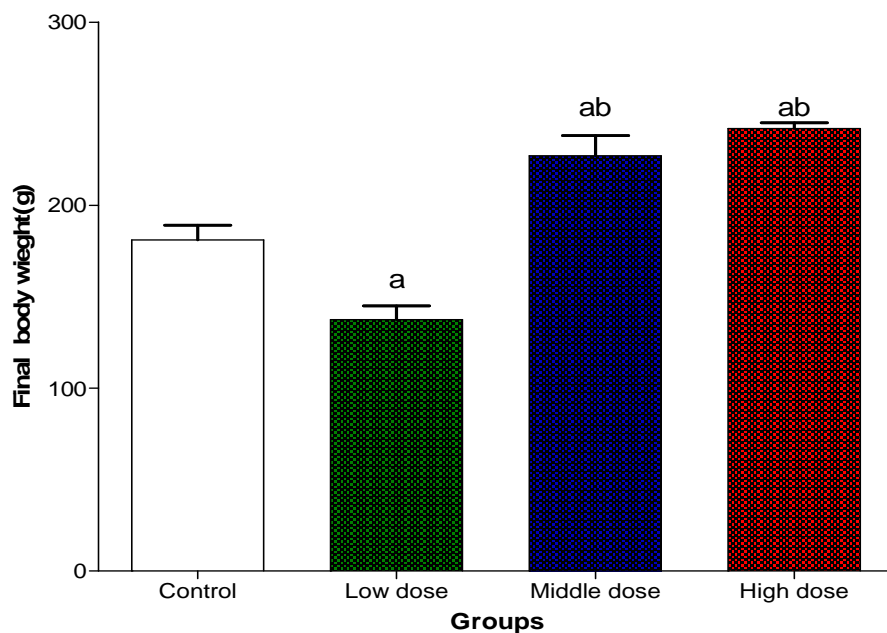


Fig 3: Final body weight Phase 1

Means \pm SEM were plotted, a = significantly different from control ($p < 0.05$), ab = significantly different from control and low dose ($p < 0.05$).

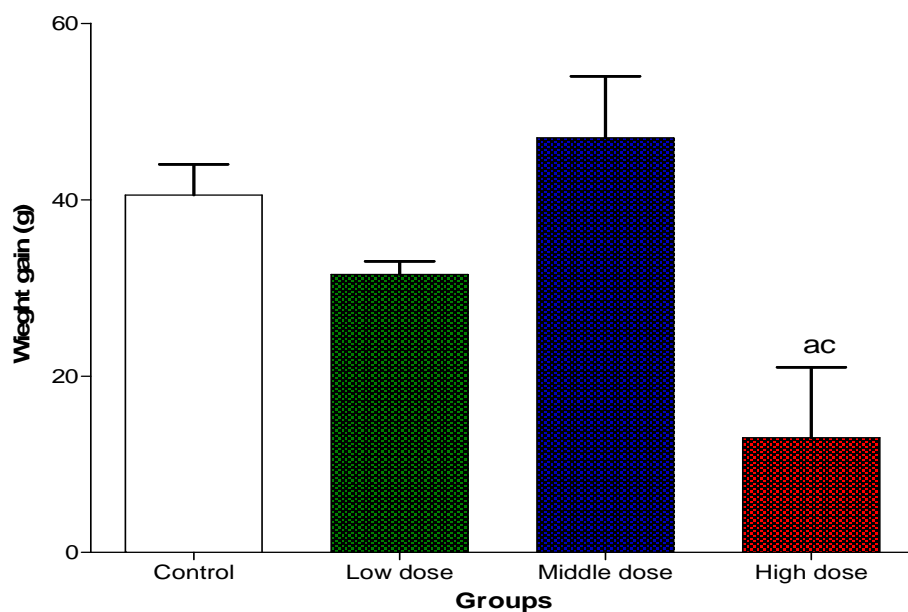


Fig 4: Weight gain Phase 1, Means \pm SEM were plotted, ac = significantly different from control and middle dose ($p < 0.05$),

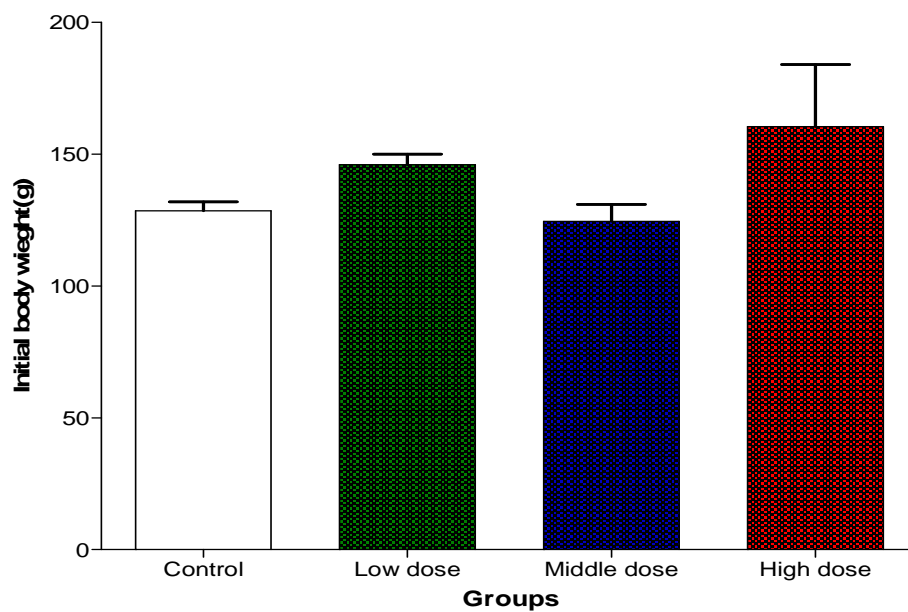


Fig 5: Initial body weight in Phase 2, Means \pm SEM were plotted

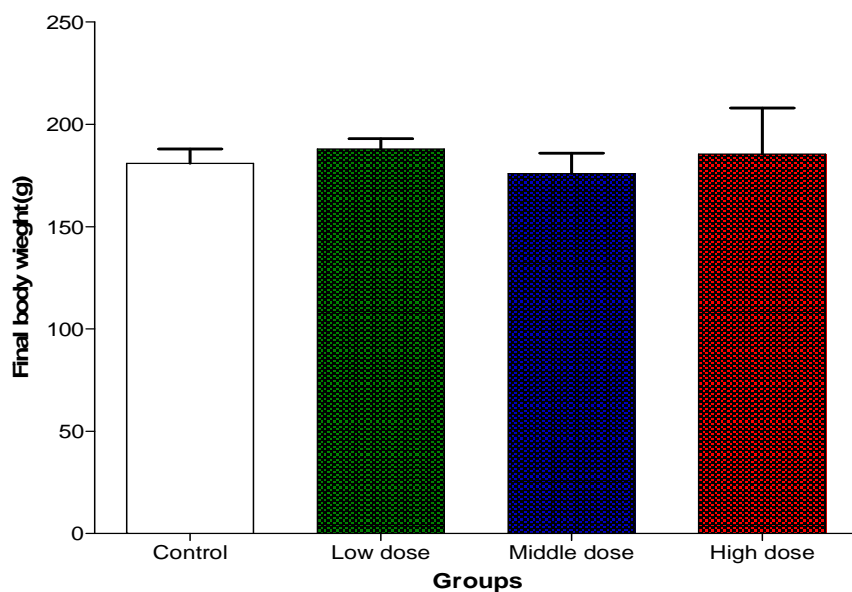


Fig 6: Final body weight Phase 2, Means \pm SEM were plotted

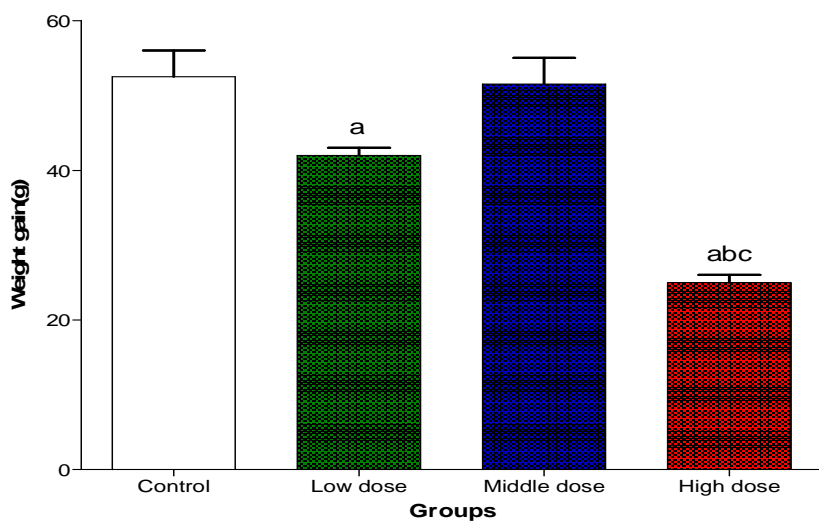


Fig 7: Weight gain Phase 2, Means \pm SEM were plotted

Histopathological Findings

Control Group Phase -1

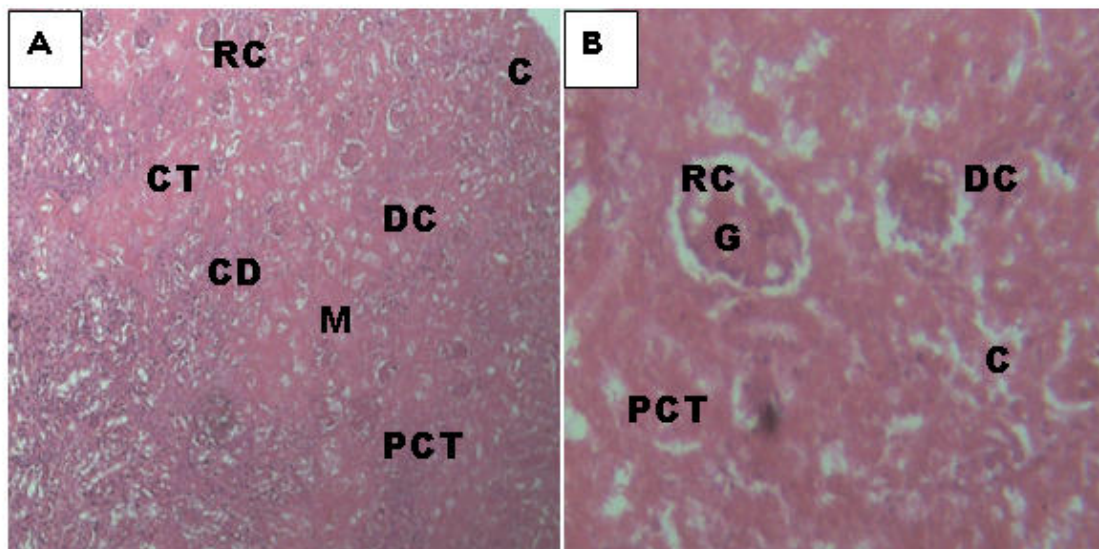


Plate 1 Photomicrograph of the neonatal Kidney tissue without treatment at Magnification A (X100) and B (X400) stained with H and E technique

Keys: RC –Renal corpuscle, G- Glomerulus PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL –Squamous epithelial lining, M – Medulla and C- Cortex.

PHASE-1- LOW-DOSE

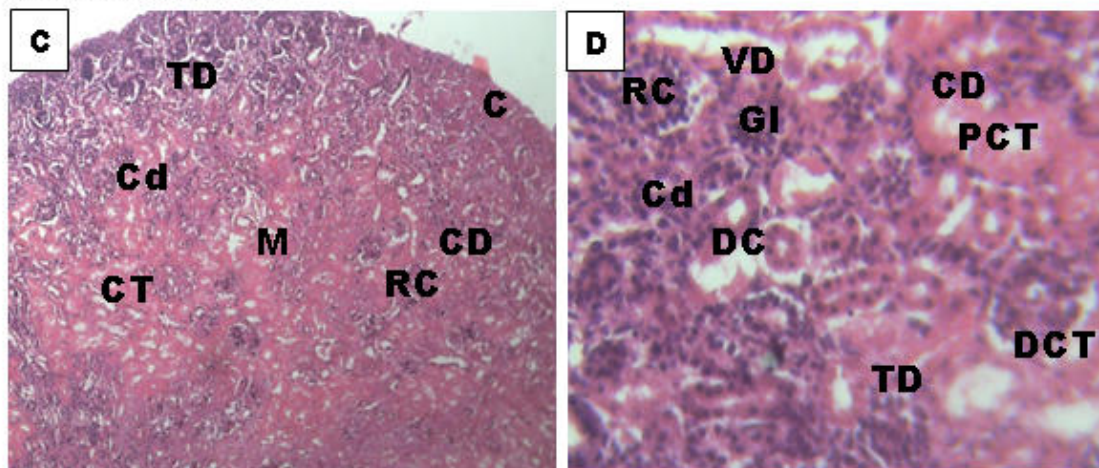


Plate 2 Photomicrograph of the neonatal Kidney tissue treated with 500mg/kg of Nauclea latifolia at Magnification C(X100) and D(X400) stained with H and E technique

Keys: RC –Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL –Squamous epithelial lining, M – Medulla, C- Cortex, Pn- Pyknotic nucleus,

PHASE-1 -MIDDLE -DOSE

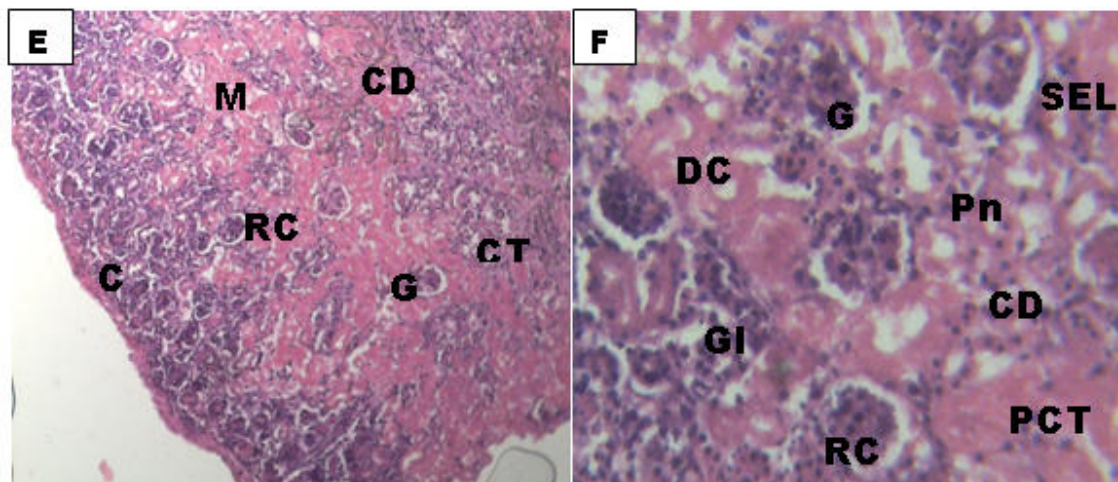


Plate 3 Photomicrograph of the neonatal Kidney tissue treated with 1000mg/kg of Nauclea latifolia at Magnification E(X100) and F(X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex and Pn- Pyknotic nucleus

PHASE-1- HIGH-DOSE

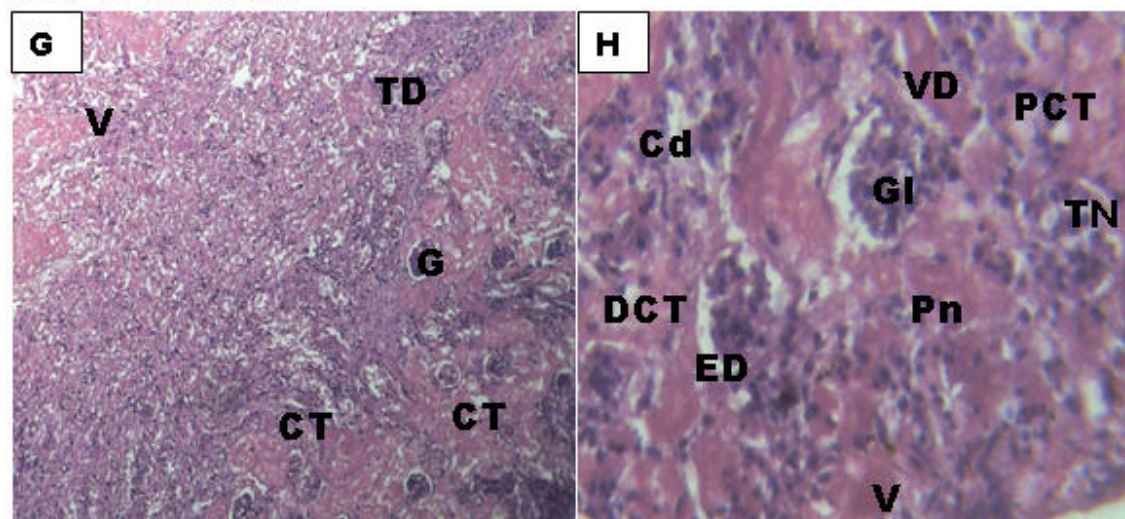


Plate 4 Photomicrograph of the neonatal Kidney tissue treated with 1500mg/kg of Nauclea latifolia at Magnification G(X100) and H (X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, ED -Epithelia lining degeneration, M - Medulla, C- Cortex, Pn- Pyknotic nucleus, VD - Vascular degeneration, TN - Tubular necrosis and Cd- Cellular degeneration.

PHASE -2- CONTROL-GROUP

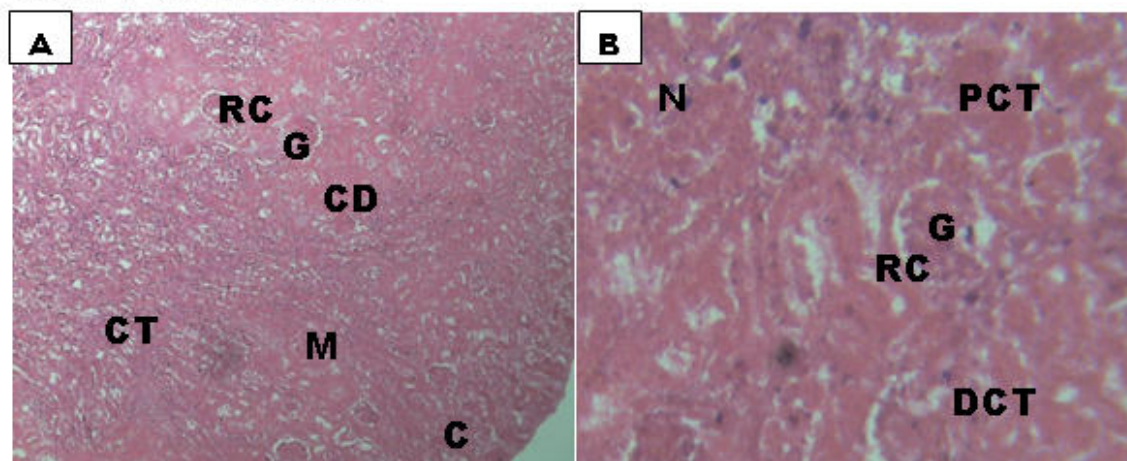


Plate 5 Photomicrograph of the neonatal Kidney tissue without treatment at Magnification A (X100) and B (X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla and C- Cortex.

PHASE-2- LOW DOSE

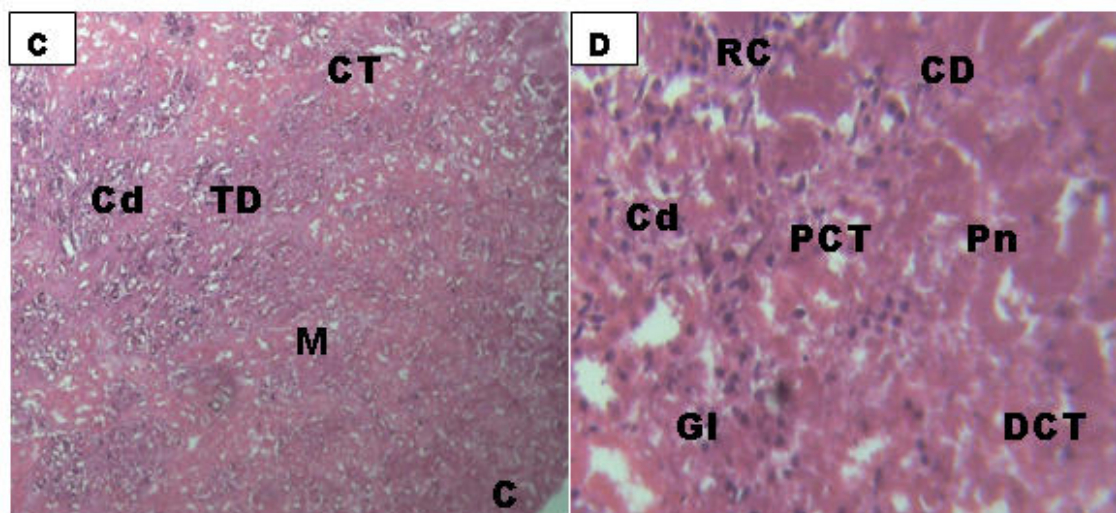


Plate 6 Photomicrograph of the neonatal Kidney tissue treated with 500mg/kg of Nauclea latifoli at Magnification C (X100) and D(X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex, Pn- Pyknotic nucleus, TD - Tubular degeneration and Cd - Cellular degeneration.

PHASE-2-MIDDLE -DOSE

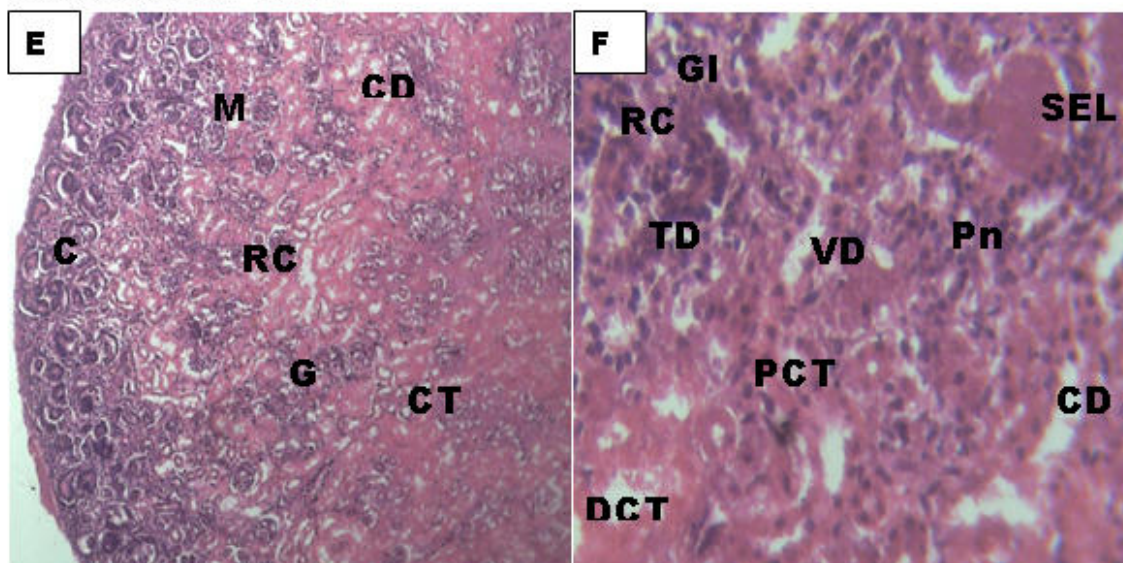


Plate 7 Photomicrograph of the neonatal Kidney tissue treated with 500mg/kg of *Nauclea latifolia* at Magnification E(X100) and F(X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex, Pn- Pyknotic nucleus. TD - Tubular degeneration. Cd - Cellular degeneration and VD - Vascular degeneration.

PHASE-2-HIGH-DOSE

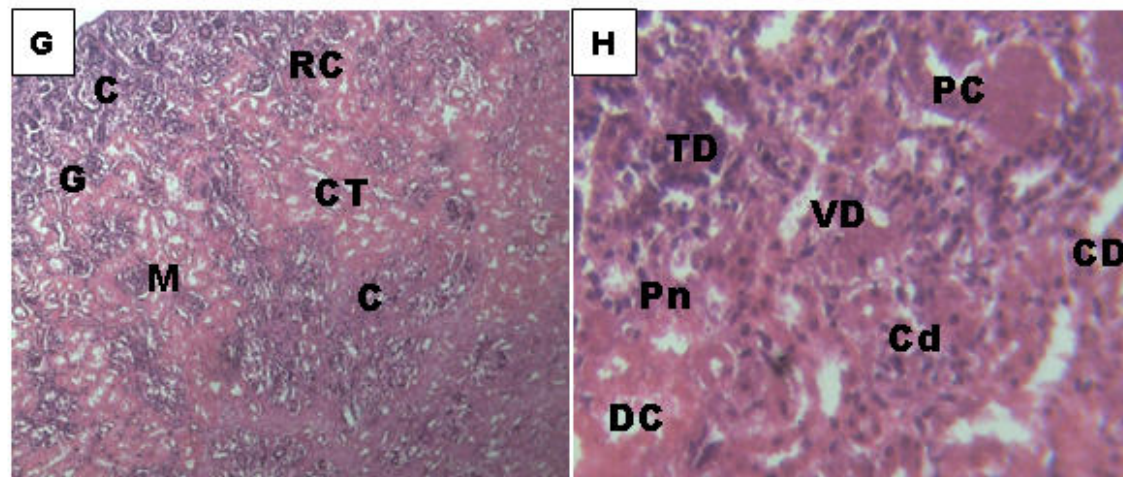


Plate 8 Photomicrograph of the neonatal Kidney tissue treated with 1500mg/kg of *Nauclea latifolia* at Magnification G(X100) and H(X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex, V- Vacuolation, Pn- Pyknotic nucleus

PHASE 3- CONTROL-GROUP

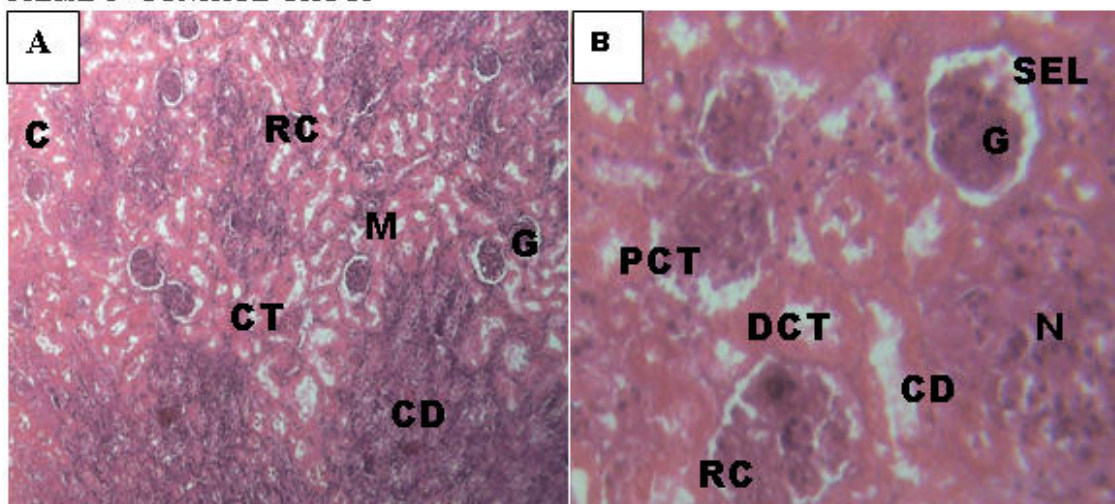


Plate 9 Photomicrograph of the neonatal Kidney tissue without treatment at Magnification A (X100) and B (X400) stained with H and E technique

Keys: RC -Renal corpuscle, G- Glomerulus PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla and C- Cortex.

PHASE-3-HIGH-DOSE

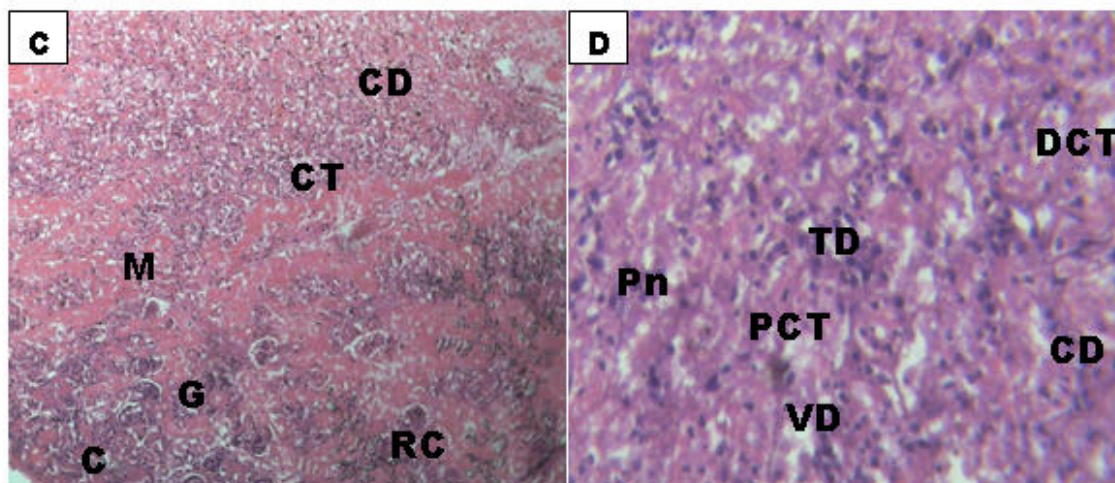


Plate 10 Photomicrograph of the neonatal Kidney tissue treated with High dose ,1500mg/kg of Nauclea latifolia at Magnification G(X100) andHT(X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex, V- Vacuolation, Pn- Pyknotic nucleus, VD - Vascular degeneration and TD - Tubular degeneration.

PHASE- 3- MIDDLE- DOSE

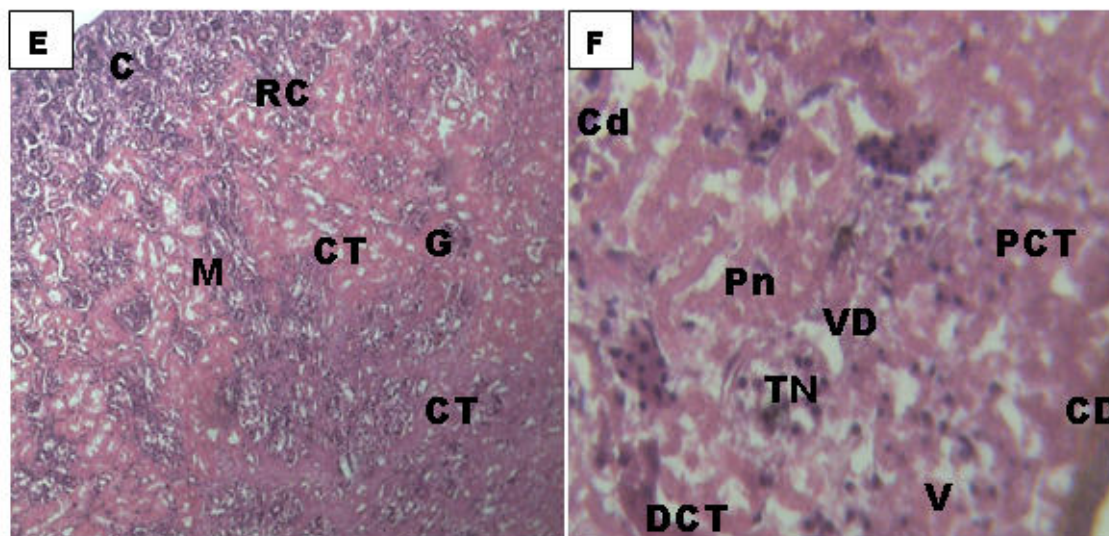


Plate 11 Photomicrograph of the neonatal Kidney tissue treated with 1000mg/kg of *Nauclea latifolia* at Magnification E (X100) and F (X400) stained with H and E technique

Keys: RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex, Pn- Pyknotic nucleus,

PHASE-3-HIGH-DOSE

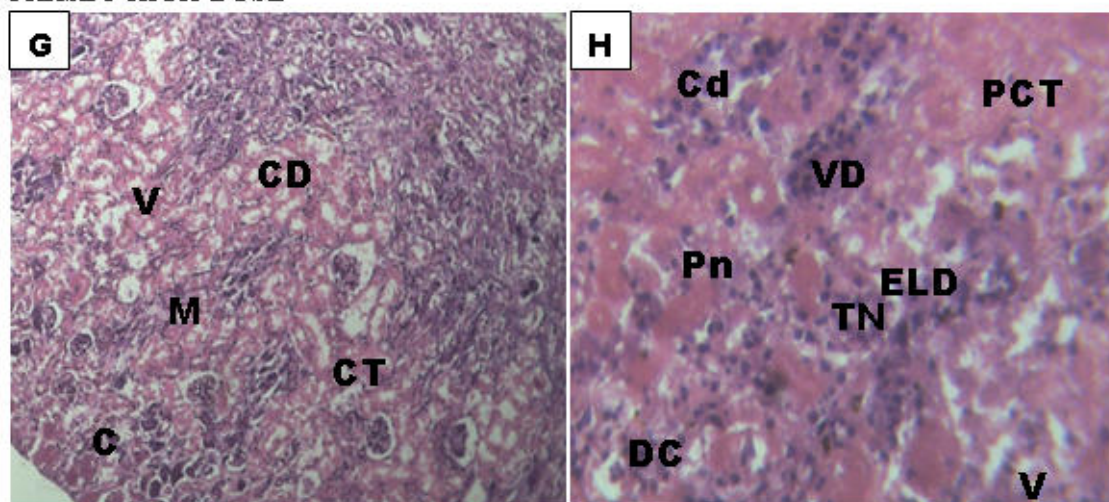


Plate 12 Photomicrograph of the neonatal Kidney tissue treated with 1500mg/kg of *Nauclea latifolia* at Magnification G(X100) and H(X400) stained with H and E technique

Keys:RC -Renal corpuscle, GI- Glomerular inflammation PCT- proximal convoluted tubules, DCT- Distal convoluted tubules, G- glomerulus, SEL -Squamous epithelial lining, M - Medulla, C- Cortex, Pn- Pyknotic nucleus, V- Vacuolation, VD - Vascular degeneration and TN - Tubular necrosis.

PHASE 1

CONTROL GROUP 1 - Plate 1 A(X100) and B(X400) of control neonatal Kidney tissue without treatment revealed normal cellular pattern with marked cortex, medulla, renal corpuscle containing glomerulus, collecting duct, convoluted tubules, all within normal cellular profile, without abnormality.

GROUP 2 Plate 2 C(X100) and DC(X400) of neonatal Kidney tissue treated with low dose 500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation and tubular degeneration as compare to control group.

GROUP 3 Plate 3 E(X100) and F(X400) of neonatal Kidney tissue treated with middle dose 1000mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation and tubular degeneration as compare to control group.

GROUP 4 Plate 4 G(X100) and H(X400) of neonatal Kidney tissue treated with High dose 1500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation and tubular degeneration as compare to control group.

PHASE 2

CONTROL GROUP 1 - Plate 5 A(X100) and B(X400) of control neonatal Kidney tissue without treatment revealed normal cellular pattern with marked cortex, medulla, renal corpuscle containing glomerulus, collecting duct, convoluted tubules, all within normal cellular profile, without abnormality.

GROUP 2 Plate 6 C(X100) and D(X400) of neonatal Kidney tissue treated with low dose 500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation and tubular degeneration as compare to control group.

PHASE -3 (GROUP 2) Plate 7 E(X100) and F(X400) of neonatal Kidney tissue treated with middle dose 1000mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation and tubular necrosis as compare to control group.

GROUP 4 HIGH - DOSE Plate 8 G(X100) and H(X400) of neonatal Kidney tissue treated with High dose 1500mg/kg of *Nuclea latifolia* revealed abnormal cellular pattern with numerous area of tubular necrosis as compare to control group.

PHASE 3

CONTROL GROUP - Plate 9 A(X100) and B(X400) of control neonatal Kidney tissue without treatment revealed normal cellular pattern with marked cortex, medulla, renal corpuscle containing glomerulus, collecting duct, convoluted tubules, all within normal cellular profile, without abnormality.

GROUP 2 LOW- DOSE - Plate 10 C(X100) and D (X400) of neonatal Kidney tissue treated with low dose 500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation, tubular and vascular degeneration as compare to control group.

GROUP 3 MIDDLE-DOSE Plate 11 E(X100) and F(X400) of neonatal Kidney tissue treated with middle dose 1000mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with area of glomerular inflammation, tubular necrosis and vascular degeneration as compare to control group.

GROUP 4 HIGH-DOSE- Plate 12 G(X100) and H(X400) of neonatal Kidney tissue treated with high dose 1500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern with numerous area of tubular necrosis and vascular degeneration as compare to control group.

DISCUSSION

Gross Morphological Study

Over the years medicinal plant extracts have been shown to contain substances of therapeutic significance (Valnet, 1994). Even though medicinal plants are effective in healing of various health disorders, continuous use of these herbal plants can in turn lead to a plethora of side effects (Valnet, 1994). Morphologically, comparing the weight of the animals before and after treatment in phase 1, 2 and 3 revealed a significant difference in groups administered low dose, middle dose and high dose at ($p < 0.05$) compared to the control. In phase 1 there was weight gain between the control groups and experimental groups, Low dose, middle dose and high dose group. The weight gain seen in middle dose and high dose groups were significantly less than the control and low dose while that of the low dose group was significantly less than control at ($p < 0.05$). The weight in group treated with high dose of the extract was significantly less than the control and middle dose but insignificantly different from low dose at ($p < 0.05$). Phase 2 showed weight gain. Animals treated with high dose of the extract showed a significant lesser weight gain compared to the control, low dose and middle dose while in Phase 3, there was weight gain in the experimental groups and control groups. Experimental groups administered with Low dose of the extract showed a lesser weight gain compared to the control while the group treated middle and high dose of the extract showed a significant difference at ($p < 0.05$) compared to the control, indicating potentials of the extract to support weight gain at the dosage given.

Histopathological changes

The role of Histopathology in drug evaluation and toxicity cannot be overemphasized, it has been the major approach to underline the basic abnormalities or alteration seen in tissue after been exposed to extracts or agents, as clearly seen in Phase 1,2 and 3, Plate 2,6 and 10, the neonatal Kidney tissue treated with low dose 500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern, glomerular inflammation and tubular degeneration, Plate 3,7 and 11 of neonatal Kidney tissue treated with middle dose 1000mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern, glomerular inflammation and tubular degeneration while Plate 4,8 and 12 of the neonatal Kidney

tissue treated with High dose 1500mg/kg of *Nauclea latifolia* revealed abnormal cellular pattern, glomerular inflammation necrosis and tubular degeneration as compare to control group in plate 1, 5 and 9 respectively. The repeated administration of *Nauclea latifolia* at varying doses brought about decrease in urea and cholesterol levels implying liver dysfunction and compromise of secretory and excretory functions of the kidney (Arise *et al.*, 2012). It has been reported that the extract of *Nauclealatifolia* causes significant changes in alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in various tissues studied, suggesting that, the prolong administration of the extract at various doses may cause damage to hepatic and renal cells as well as disrupt amino acid metabolism (Arise *et al.*, 2012).

CONCLUSION

From the result obtained, there was weight gain in body weight of all the groups compared to their controls suggestive the ability of the extract to potentiate and accelerate weight increase. Obviously, the use of *Nauclea latifolia* during gestation showed significant histopathological changes in all the groups and Phases. These suggest that care should be taken in the use of this plant for treatment during gestation for reasons of its counterproductive consequences resulting possibly from the inflammation, degeneration, distortion of the cyto-architecture and necrosis of the neonatal kidney. Though *Nauclea latifolia* have been demonstrated been useful in the treatment of various diseases such as malaria, filariasis, diarrhoea and intestinal abnormalities. However the intake during gestation could pose deleterious and histopathological alterations in the neonatal kidney, thus the use of this plant should be restricted to a pregnancy free state.

CONFLICT INTERESTS

The authors declared that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All the Authors contributed equally.

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