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Teratogenic Effects of *Lerhacacig* on the Developing Kidney and Liver of Wistar Rat Fetuses (*Rattus Norvergicus*)

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Abstract:- Herbal products are frequently regarded as "safe" by the general public, but not without side effects. The aim of this study was to assess the probable teratogenic effects of Lerhacacig on the developing wistar rat foetuses. Lerhacacig, a local herbal concoction, made up of five different plant products (CP, GL, RB, CL, LC), is consumed mostly by the south western (yoruba) women in Nigeria during pregnancy. 25 adult female Wistar rats weighing between 150-200g were randomly assigned to three (3) experimental groups and a control group. The experimental groups consisted of three groups viz: group 1 (CP + GL + RB – T1); Group 2 (LC + CL- T2); Group 3 (RB + CP + CL + LC +GL- T3). All animals were given 0.8ml/kg body weight of their respective extract daily by oral administration for 21days. They were sacrificed on the 21st day of pregnancy using light anesthesia. The Neonatal weight, litter size, crown rump length, head circumference, femur length and humeral length anthropometric measurements were taken for both experimental and control groups respectively. The kidney and liver were harvested, weighed and processedfor routine and special histological analyses.

The results from this present study revealed that 1fetus from group 3 mothers presented with unilateral renal agenesis. Another mother in the same group also presented a case of foetal resorption in which up to six resorption sites were observed. There was no significant difference in the crown rump length, femur length, humeral length and head circumference across the groups. However, the neonatal weight was significantly different across the groups. The photomicrographs of organs harvested from the experimental groups of Lerhacarcig also showed some pathologic changes while those of the control appeared normal. In conclusion, the combined extract of lerhacacig could be considered teratogenic as observed in this study. For further study, it is pertinent to isolate each plant in order to elucidate which root was responsible for the teratogenic effects as this present study only suggested a combinatorial effect of the extracts as against individualizing the plant.

GLOSSARY: CP- Cissus Populnea, GL- Gongronema Latifolium, RB- Rhaphiostylis Beninesis, CL- Carpolobia Lutea, LC- Lecaniodiscus Cupanioides, T1- 1ST trimester, T2- 2ND trimester, T3- 3RD trimester.

Keywords:- *Teratogenicity, Lerhacacig, Resorption and unilateral Renal Agenesis.*

I. INTRODUCTION

The controversy surrounding the causes of adverse pregnancy outcomes especially congenital anomalies and stillbirth have been the subject of many studies in recent years, Preterm birth is a major public health problem all over the world, but little is known about the size of this problem in developing countries.[1]

Birth defects are structural or functional abnormalities present at birth that can cause physical disability, intellectual and developmental disability(IDD), and other health problems [8]Some may be fatal, especially if not detected and treated early. At present, the etiology of congenital malformations can be divided into three categories: unknown, genetic, and environmental. Unfortunately, the largest group (65-75%) has an unknown etiology, whereas the most common known cause is genetic (15-25%)[5][12][17] .It is estimated that approximately 10-15% of congenital structural anomalies are the result of the adverse effects of environmental factors on prenatal development [7]. This means that approximately 1 in 250 newborn infants have structural defects caused by an environmental exposure and, presumably, a larger number of children have growth retardation or functional abnormalities resulting from nongenetic causes, in other words, from the effects of teratogens. A teratogen is any environmental factor that can produce a permanent abnormality in structure or function, restriction of growth, or death of the embryo or fetus [10]Exposure to teratogens will affect the fetus or embryo based on the following factors:

- The duration of exposure
- The amount of teratogenic substance
- The stage of development of the fetus during the exposure [3]

Teratogenic exposures during prenatal development cause disruptions regardless of the developmental stage or site of action. Most structural defects caused by teratogenic exposures occur during the embryonic period, which is when critical developmental events are taking place and the foundations of organ systems are being established. Different organ systems however, have different periods of susceptibility to exogenous agents [11]

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Herbal medicines may be used sometimes as part of maternal care to treat pregnancy related problems, and often to improve the well-being of the mother and/or the unborn child. The most commonly reported indications are nausea and vomiting, urinary tract infections, preparation for and/or facilitation of labor, common cold or flu, gastrointestinal problems (e.g., constipation, flatulence), pain conditions, improvement of fetal outcomes, prevention of miscarriage, relief of anxiety, treatment and/or prevention of anemia, and treatment of edema [2][9][13][15]

The name *Lerhacarcig (LRC²G)* represents the popular traditional herbal preparation consumed by pregnant women mainly in western part of Nigeria. It was coined using the letters of the botanical names of the constituent plants. In the South Western part of Nigeria, it is known as "*AGBO OLOYUN*" and serves as an alternative medicine used by most pregnant women especially the native Yoruba people. *LRC²G* comes as liquid, pastries, syrups or crushed mixtures of different parts such as the bark, leaves, stems and roots of particular plants. *LRC²*G can be soaked in a transparent container for drinking as may be specified by the traditional healthcare giver. It can also be boiled for drinking or bathing depending on the prescription by the expert on the ailment that is being treated.

The main purpose of this scoping review is to inform the public regarding probable effects of the herbal concoction (*lerhacacig*) on the developing fetus.

II. ANTHROPOMETRIC MEASUREMENTS

A. FEMUR LENGTH

Femur length is the distance from the head of the femur to its distal end. It is one of the most crucial measurements, as it not only serves to help gauge fetal growth but is also used to help indicate if there is a possibility that the baby has skeletal dysplasia, Down syndrome, dwarfism, and other possible abnormalities.

B. CROWN RUMP LENGTH:

Crown-rump length (CRL) is the measurement of the length of human embryos and fetuses from the top of the head (crown) to the bottom of the buttocks (rump). It is typically determined from ultrasound imagery and can be used to estimate gestational age[19]

C. HEAD CIRCUMFERENCE (HC):

It is a measurement of a child's head around its largest area. It measures the distance from above the eyebrows and ears and around the back of the head.

Measurement of the head circumference is an important part of routine well-baby care. During the well-baby exam, a change from the expected normal head growth may alert the doctor of a possible problem. For example, a head that is larger than normal or that is increasing in size faster than normal may be a sign of several problems, including water on the brain (hydrocephalus).A very small head size (called microcephaly) or very slow growth rate may be a sign that the brain is not developing properly[16]

III. MATERIALS AND METHODS

Animals: Forty adult Wistar rats (Rattus novergicus) weighing between 150 and 250 g were procured for this study, Out of which twenty five (25) of them where females and fifteen (15) males. 5females were randomly distributed in cages. The animals were kept in the Animal Holding of the Faculty of Basic Medical Sciences, department of Anatomy, O.O.U. Unnecessary restraint and overcrowding was avoided. Food and water were made available ad libitum. Beddings in the cages was ensured to be non-allergic, dust-free, inedible, absorbent, non-toxic and free of pathogenic organisms. The bedding was changed twice a week to prevent the build-up of pathologic ammonia levels. Rats were properly fed to ensure adequate growth and development and prevent malnutrition-induced disease conditions.

A. COLLECTION OF PLANTS, IDENTIFICATION AND VOUCHERING

The ethno-botanical data (local name, mode of preparation and medicinal uses) was collected through Interviews and discussion with the traditional "AGBO" dealer using a more qualitative interesting technique with the help of a Yoruba speaking friend.

Discussion with the traditional "Agbo" dealer allowed descriptive response on the appropriate prescription such as part of the plant used, detached information about the mode of preparation (i.e. decocotion, paste, powder or liquid as the case may be); form of usage either fresh or dried plants and mixtures of other substances used together with the plants. The conversation was built on trust with the common goal to improve the health situation of women during pregnancy as well as the wellness of the developing fetus.

The plant materials used for the study were obtained from a traditional herbal dealer in Sagamu, Ogun state, Nigeria. The plants were taken to the University of Lagos Herbarium and where identified by the officer Mr. Oyebanji and also issued the voucher numbers.

Yoruba name	Botanical name	Voucher number
Egbo akika	Lecaniodiscus cupanioides	LUH6611
Egbo itakpara	Rhaphiostylis beninensis	LUH6777
Igi osun'sun	Carpolobia lutea	LUH6610
Igi ogboloh	Cissus populnea	(PCGLH-370).referenced
Igi Madumaro	Gongronema Latifolium	LUH6776

Table 1: Showing botanical names and voucher number of the component plants of lerhacacig

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PLANTS	PHYTOCHEMICAL CONSTITUENTS
Lecaniodiscus Cupaniodes Harbone J.B (1983)	Alkaloids, saponins, phenol, tannins, anthraquinones
Rhaphiostylis beninensis Ofeimun and Onwukeame (2006)	Anthraquinones, cardiac glycosides, flavonoids and triterpenes
Carpolobia lutea Etenbong and Nwafor(2009)	Saponins, simple sugars, terpenes and cardiac glycosides in large quantities and some flavonoids and anthraquinones.
Cissus populnea Soladoye and chukwuma (2012)	Tannins, flavonoids, saponins and steroids, alkaloids, anthraquinones, cardiac glycosides, cyanogenic glycosides.
Gongronema latifolium Nwinyi O.C *et al* (2008)	Flavonoids, terpenes, tannins, saponins and alkaloids.

Table 2: Showing phytochemical constituents present in each plant

B. PREPARATION OF LERHACARCIG EXTRACT

Appropriately weighed plants: Lecaniodiscus cupanioides planc-200g, Rhaphiostylis beninensis-200g, Carpolobia lutea-200g, Cissuss populnea-200g, Gongronema Latifolium-150g were washed and chopped into fine pieces after which they were boiled in corresponding volumes of water for 2-3hours.

C. EXPERIMENTAL DESIGN

- **Group 1-** Animals in this group received the extract of containing Cissus populnea+ Gongronema Latifolium + Rhaphiostylis given by oral administration in the 1st week representing the 1st trimester of the rat gestational period.
- **Group2-** Animals in this group received the extract of Lecaniodiscus cupanioides + Carpolobia lutea in the 2nd week representing the 2nd trimester
- **Group3-** Animals in this group received the full combination of *Lerhacacig containing* Rhaphostylis beninensis+ Cissus populnea+ Carpolobia lutea, Lecaniodiscus cupanioides, +Gongronema Latifolium in the 3rd week representing the 3rd trimester
- **Group4-** Control.

D. ANIMAL SACRIFICE AND TISSUE PROCESSING

After 21 days of pregnancy, animals were sacrificed using light anaesthesia. The kidney and liver were harvested and processedby the routine H&E histological technique for light microscopy.as well as special staining technique were carried out.

IV. RESULTS

GROUP	GROUP1	GROUP2	GROUP3	GROUP4
PARAMETER				
LITTER SIZE(n)	19	17	9	21
HC(cm)	3.90±0.07	3.93±0.06	3.85±0.08	4.02±0.05
CRL(cm)	5.11±0.17	5.30±0.17	5.69±0.18	5.44±0.13
FL(cm)	0.76±0.02	0.74±0.02	0.73±0.03	0.76±0.02
HL(cm)	0.74±0.01	0.72±0.02	0.64±0.02	0.69±0.01
MALFORMATION	-	-	+	-
RESORPTION	-	-	++++++	-
STILL BIRTH	-	-	-	-
P-N DEATH	3	3	NIL	-
FW	5.34±0.11	5.30±0.14	5.04±0.19	5.12±0.11

Table 3: Showing Litter size, Head circumference, Crown rump length, Femur length, Humeral length, Malformation, Still birth, Post-natal death and Foetal weight.of pups

	ORGAN	
GROUP	LIVER	KIDNEY
GROUP 1	0.940±0.004	0.132±0.002
GROUP 2	0.932±0.006	0.132±0.002
GROUP 3	0.936±0.006	0.144±0.009
GROUP 4	0.946±0.002	0.138±0.004

Table 4: Showing the mean weight of organs of fetuses. Data expressed as Mean±SEM.P<0.05

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V. HISTOLOGICAL ANALYSIS

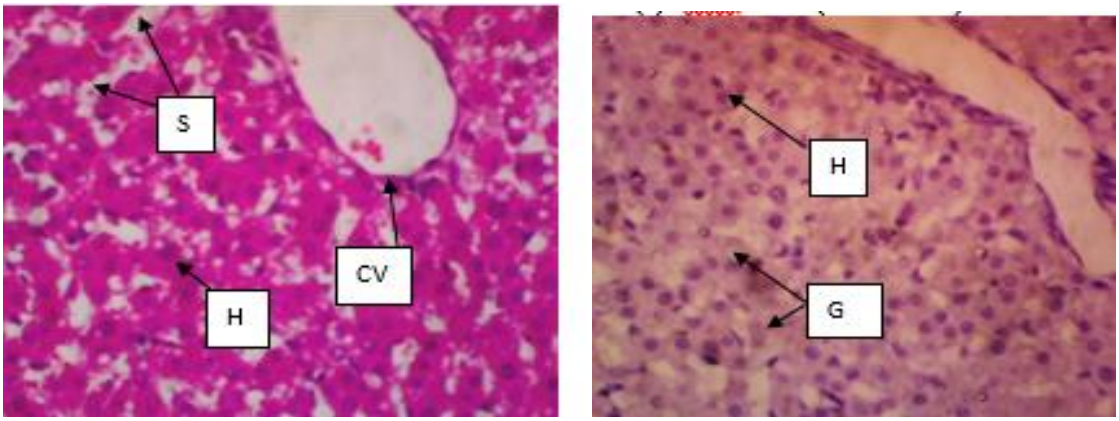

PLATE 1(a): Fetal Liver (H&E × 400) Control PLATE 1: CONTROL FETAL LIVER (PAS x400)

Fig. 1: Photomicrograph of control group(H&E) showing normal histology of the central vein, sinusoids and the radial plates of hepatic cells. Liver stained with PAS showing glycogen granules and the hepatocyte visibly

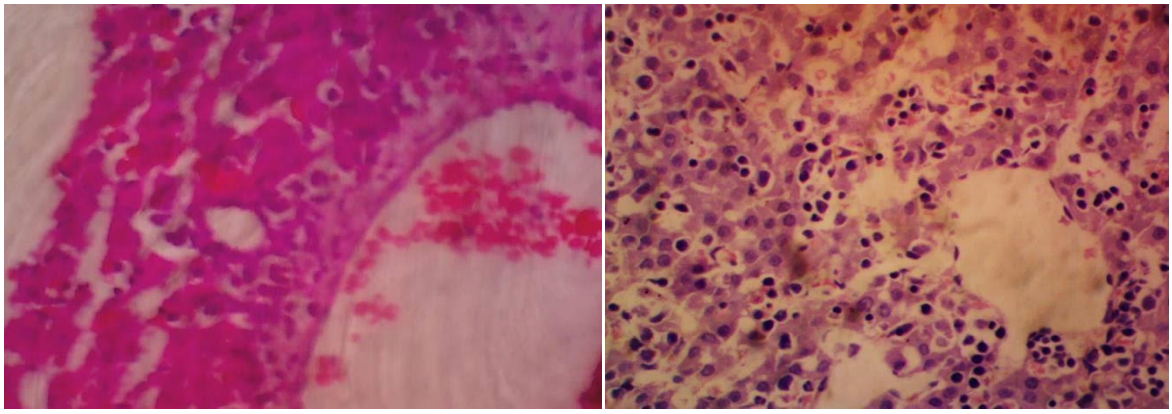

PLATE 2 (b):Fetal Liver (H&E × 400) Group 1PLATE 2(b) Fetal Liver (PAS X 400)

Fig. 2: Photomicrograph of the Group1fetal liver showing radial plates of hepatic cells appearing irregular and the nuclei is degenerated. Presence of genotoxic effect of the cells.

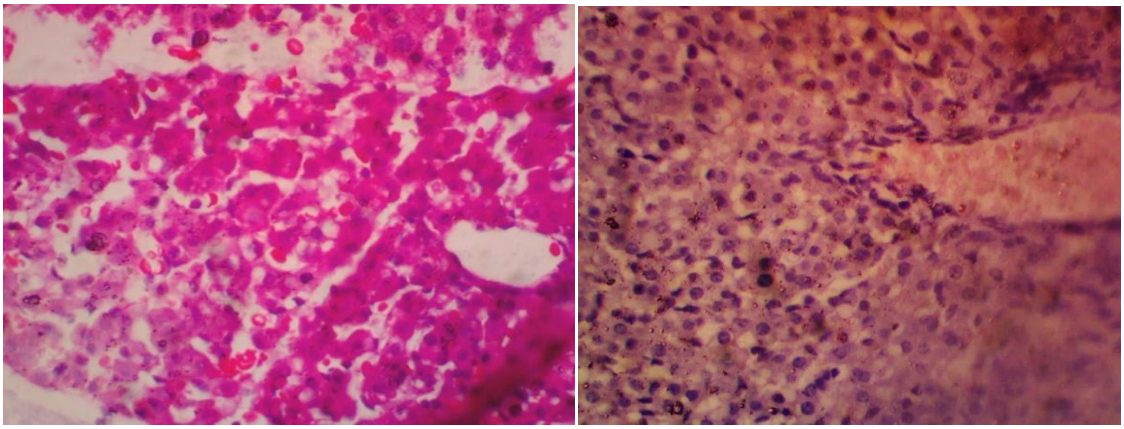

PLATE 3(c):Fetal Liver (H&E ×400) Group2 PLATE 3(c): Fetal Liver (PAS X400)

Fig. 3: Photomicrograph of the Group2 fetal liver showing more disorganised radial plates of hepatic cells, presence of red blood cells in the hepatocytes.there is a distortion of the sinuosoidal intergrity. The central vein appears smaller.

Legend
A: Red blood cells in the hepatocytes
B: shruken central vein

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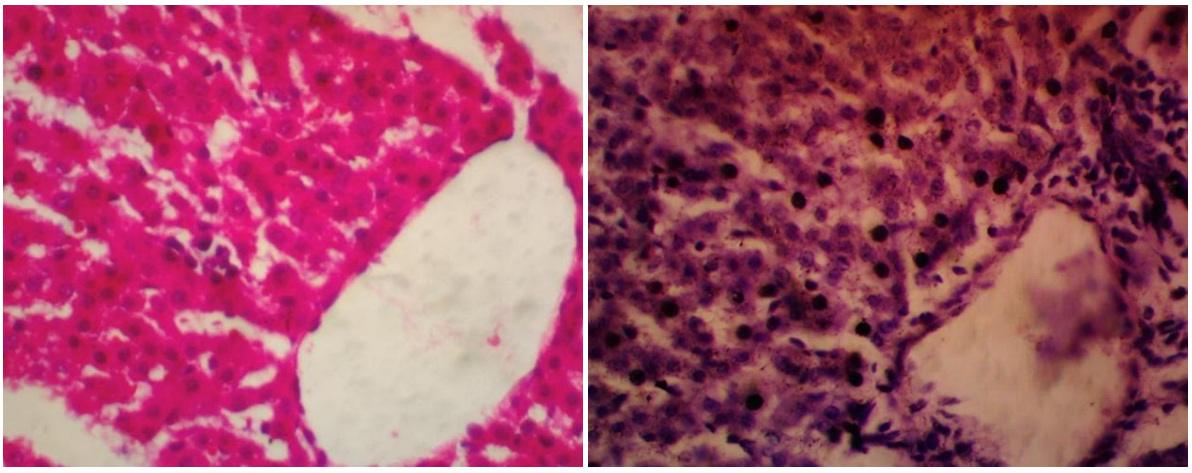

PLATE 4.(d):Fetal Liver (H&E ×400) Group2 PLATE 4.(d): Fetal Liver (PAS X400)
Fig. 4: Photomicrograph of the Group 3 fetal liver showing the central vein, the radial plates of hepatocytes appearing intact.

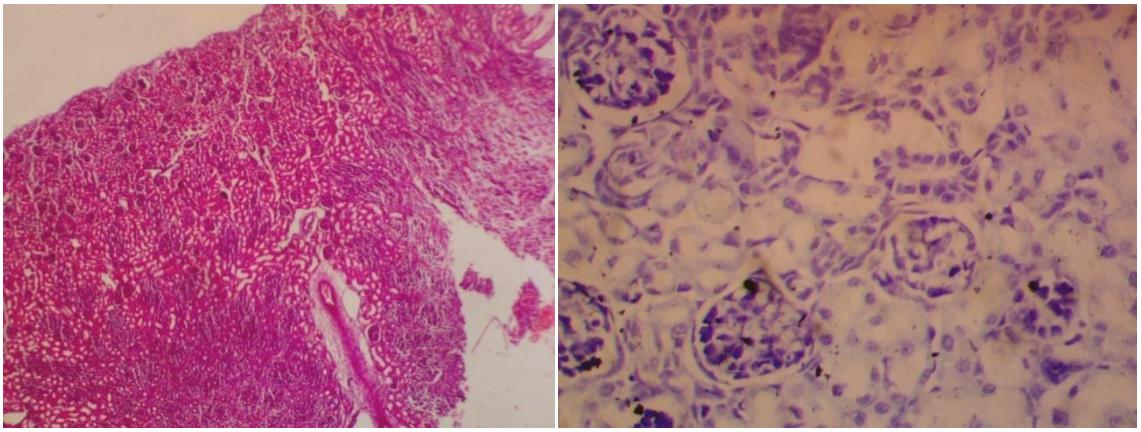

PLATE 5(a): Fetal Kidney (H&E ×40) ControlPLATE 5: CONTROL FETAL IDNEY (TOLUIDINE BLUE X400)
Fig. 5: Photomicrograph of control group showing fetal kidney (H&E X40) revealing a well outlined cortical layer.

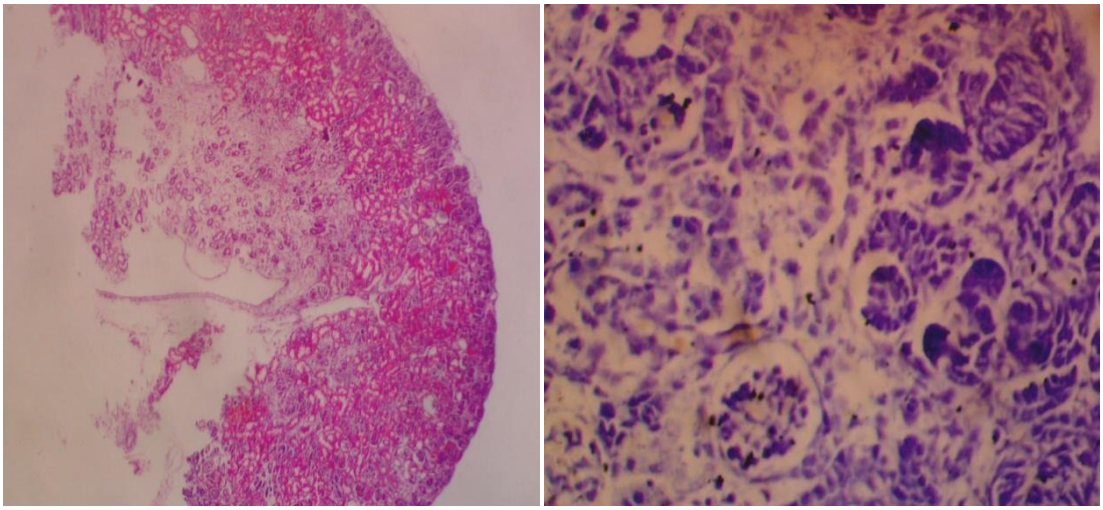

PLATE 6 (b): Fetal kidney (H&E×40) Group1PLATE GROUP1 FETAL KIDNEY (TOLUIDINE BLUEX400)
Fig. 6: Photomicrograph of group1 showing presence of diminished amount of cortical glomeruli (a) and the medulla (b).

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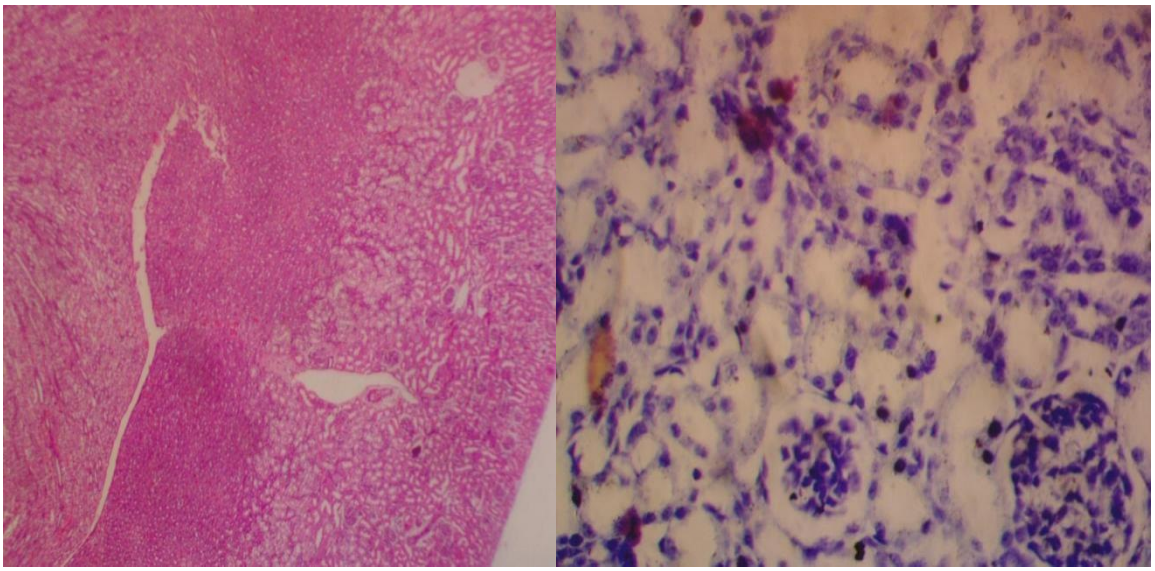

PLATE 7 (c): Fetal kidney (H&E×40) Group2PLATE 7: GROUP2 FETAL KIDNEY (TOLUIDINE BLUE X400)
Fig. 7: Photomicrograph of group3 fetal kidney showing the cortical layer appearing to have a cyst on it.

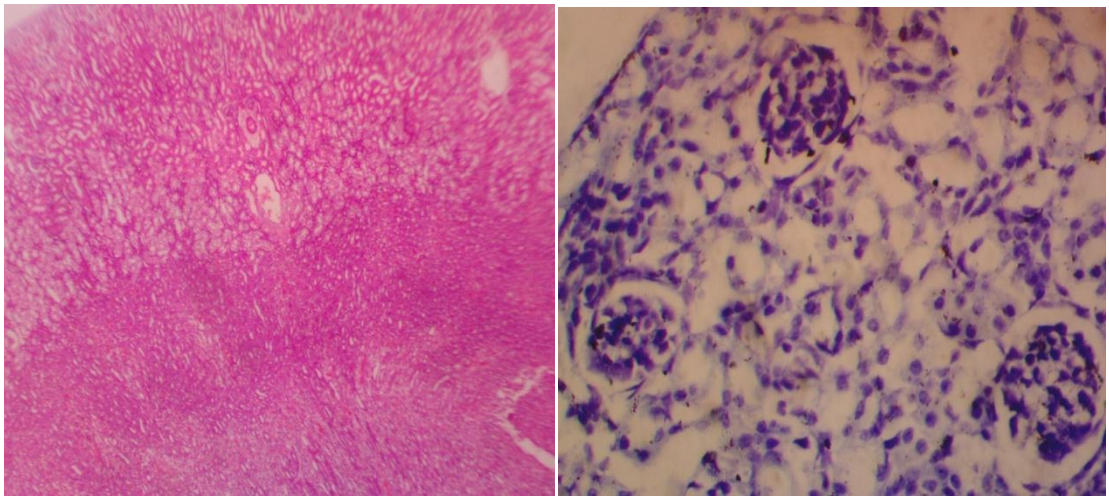

PLATE 8 (d): Fetal kidney (H&E×40) Group3PLATE 8: GROUP 3 FETAL KIDNEY (TOLUIDINE BLUE X400)

VI. DISCUSSION

The indications for the use of herbal medicines during pregnancy may vary across regions and countries, and can be mother- or child-related [13]. According to World Health Organization (2008), it was reported that all herbs are natural,but not all herbs are safe to be taken during pregnancy, Herbal medicines may be used sometimes as part of maternal care to treat pregnancy related problems, and often to improve the well-being of the mother and/or the unborn child. The most commonly reported indications are nausea and vomiting, urinary tract infections, preparation for and/or facilitation of labor, common cold or flu, gastrointestinal problems (e.g., constipation, flatulence), pain conditions, improvement of fetal outcomes, prevention of miscarriage, relief of anxiety, treatment and/or prevention of anemia, and treatment of edema.[2][9][13][15] Traditional herbal medicines are widely used compared to conventional medicine because they are readily available, cheap and are said to have a variety of effects on the fetus. and as a result of this high rise, this study was carried out.

Yoruba folklore agrees strongly with the fact that *LERHACACIG (LRC^{2}G)* otherwise known as Agbo Oloyun should be administered to every pregnant woman so as to prevent the mother from undergoing caesarean section, as such the baby is born smaller.They believe that during pregnancy the woman would be free from any form of ailment.

In this present study, rats that were administered with the 1st trimester drugs and the 3rd trimester drugs showed significant increase in weight 6.89% and 1.78% respectively in comparison with the control group at the end of the 3rd trimester. These Groups had Gongronema Latifolium as part of the herb administered in the respective trimesters. Considering the litter size, mothers in the group3 recorded the lowest number of litter and with lowest mean weights than other litters.

LERHACACIG (LRC^{2}G) did not have any significant effect on the fetuses of groups 1and 2, but in group 3, a

malformation was observed in one of the pup which was presented with Unilateral Renal Agenesis (URA). 1 male pup out of 9 presented with this case which is synonymous to the incidence of unilateral renal agenesis. URA occurs when the uretic bud (kidney bud) fails to make contact with the metanephric blastema during kidney formation, thereby leading to the absence of one kidney[14]. This malformation is said to be compatible with life. In this group also, six resorption sites were observed in one of the mother at the time of sacrifice.

The Crown rump length serves as another indicator for gestational age. In this study, there was no significant increase in the Crown rump length of the pups between and within groups. This indicates that there was a normal growth across the axial skeleton during pregnancy. this result agreed with Bassett *et al*, 2008, which reported that it is only an intact hypothalamic-pituitary- thyroid axis which regulates the skeletal development through the actions of thyroid hormone which are expressed in chondrocytes and osteoblast[4]. No significant increase in the Head circumference of fetuses was seen in this study revealing that there was no adverse effect of the drugs on the fetuses.this depicts that there was no case of Hydrocephaly. Fetal weight is an important parameter for assessing fetal growth and development, in this study the fetal weight was significant, there was no intra uterine growth retardation. In this study there was a significant increase in the femur length and humeral length of pups between and within the groups. This is an indicating factor that lerhacacig did not have an interference with the Endoskeletal system during the formative stage. Five pups from each groups were selected and their organs were properly weighed. Concerning other observations, there was no case of Omphalocele, Gastroschisis and cleft palate seen in the pups between and within the groups. Histological study of the liver across all the treatment groups revealed no detrimental effect of the drug on the organ using the Haematoxylin and Eosin stain.

On using the PAS stain, it also revealed the presence of the nuclei and the glycogen. On histological examination of the kidney, Group 3 showed presence of cyst in the cortical layer. it therefore explains that there is need to do a deeper study on the kidney using both extract. On stainning with the toluidine blue, there was no show of detrimental effect to the basement membrane except in Group 2 that had glomeruli damage.

VII. CONCLUSION

The findings of this study provides baseline information on the teratogenic effect of lerhacacig on the kidney of a developing wistar rat .

AUTHORS CONTRIBUTION

ME wrote the introduction, acquired the herbs and took it for authentication at the herbarium, ME was responsible for the preparation of the extract as instructed the trado medical health service provider. OG wrote the Literarture review, prepared the materials and method , OG purchased the wistar rats, fed, harvested, weighed the Organs of the rats . HB wrote the discussion and the conclusion.

CONFLICTS OF INTEREST

There was no conflict of interest amongst the authors.

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