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Effect of Aqueous Extract of Cola Nitida on Normal Development of Distal Gastrointestinal Tract

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ABSTRACT

Normal and abnormal development of anorectum is still up for debate and the etiology of abnormal development of anorectum is poorly understood. Caffeine is a vasoconstrictor that has been implicated in abnormal anorectal development and is a major constituent of Kola nut (Cola nitida). Kola nut is consumed heavily by pregnant women in Nigeria to control vomiting. We examined the teratogenic effects of oral administration of the aqueous extract of Cola nitida (AECON) on fetal growth and development of distal gastrointestinal tract in Wistar rats. Twelve pregnant Wistar rats were used for this study and were divided into the Control group that received distilled water and experimental groups E1, E2 and E3 that received 200mg/kg, 400mg/kg, 600mg/kg of AECON respectively and orally throughout the period of pregnancy. Gross and microscopic examinations of the pups recovered were conducted to observe the various external and internal congenital malformations in the different groups. Recorded data were analysed using one-way ANOVA followed by Dunnette test. Values were considered statistically significant at $p<0.05$. There was a progressive reduction in the number of litters with increasing dosage of AECON in the experimental groups. Also, the weight of the pups in the experimental groups were reduced in comparison with the control group. None of the pups in all the groups had anorectal malformations or other associated congenital anomalies. Histopathological and biochemical changes were evident following administration of the extract. Based on the result of the study, oral administration of AECON during pregnancy does not have teratogenic effect on the rats but has adverse effects on fetal development and also affected biomarkers of liver damage. Therefore, the safety of the extract during pregnancy cannot be assured.

1. Introduction

Normal and abnormal development of the hindgut is still in debate (Kluth, *et,al, 2011).* Despite many efforts, the embryology and pathology of numerous congenital anomalies in humans is still a matter of speculation because of the following: a shortage of study material (both normal and abnormal embryos), various technical problems involved in the study of development of embryo, misconceptions and/or outdated theories concerning normal and abnormal human embryology (Kluth,2010). The etiology of abnormal development of anorectum is multifactorial with genetic (Kong and Singh, 2008) and environmental factors suggested as the major causes (Yuan, 2005). The evidence for this is still scarce as most potential risk factors were rarely reported (Charllotte, 2010). Of the environmental factors, drugs and chemical teratogens that have been reported in the abnormal development of the anorectum includes: ethylene thiourea, Adriamycin and caffeine in laboratory animals **(**Adebayo, 2012)**.** *Cola nitida* (Kola nut) is the seed-pod of an evergreen tree belonging to the family of Sterculiaceae (Cocoa) that is native to Africa (Blade,2000). Caffeine is the main constituent of Kola nut and its majorly responsible for its physiological activities *(*Umoren *et, al, 2009).* In recent years, concerns have been raised about the possible teratogenic

effects of *Cola nitida* as its consumption before conception and during the early pregnancy has been implicated as a risk factor for spontaneous abortions (Oyedeji, 2012) and delayed conception (Agbai and Ugwu,2012).

2. Objectives of the Study

The objectives of this study were to investigate if maternal consumption of *Cola nitida* in early pregnancy has teratogenic effects on the process of development of the anorectum of Wistar rat embryos, investigate any other associated anomalies that can be induced by aqueous extract of *Cola nitida* and to investigate the phytochemical profile of aqueous extract of *cola nitida.*

3. Research Questions

The study seeks to find answers to the following questions:

a) Does the maternal consumption of kola nut during pregnancy has teratogenic effects on the process of development of anorectum of Wistar rats?
b) Does oral administration of aqueous extract of *Cola nitida* causes significant histomorphology changes or variation in anorectum of fetuses of wistar rats?
c) What are the various constituents of *cola nitida*?

4. Literature review

There are several theories put forward to explain Anorectal Malformation by researchers, persistence of the anal membrane after seven and a half weeks can explain imperforate anus and anal stenosis. (Larsen, 2001). Anorectal malformations can also develop as a result of defect of the cloacal plate between the hindgut and tail groove (Ikebukuro and Ohkawa,1994). Absence of the tail groove and maldevelopment of the dorsal cloacal membrane, cloacal configuration, and urorectal septum are likely to also be responsible for the formation of anorectal malformation (Bai et, al,2004). Maldevelopment of the septum may also lead to congenital defects in which the urethra, vagina, and rectum fuse to form a common channel that opens with a single orifice (Pena and Kessler, 1998). Deformities of the cloacal membrane may also form the basis of congenital anorectal malformations (VanderPutte,1986). Despite all these attempts at explaining ARMs, there is no universally accepted theory to explain anorectal embryology and the abnormal development that produces ARM. Therefore, the hindgut continues to be a source of controversy in gastroenterology (Bai *et, al*,2004). Of all the ARMs, Imperforate anus is the most marked appearance of anorectal anomalies and it is identified during or shortly after birth. Characterization of teratogenic exposures involves the specific agent, the dose of the agent, the gestational age (critical period), and other factors such as genetic susceptibility (Friedmann *et, al*.,2002). Recently a number of animal models have been deployed to study the background of ARM. In these models, these following agents were used:

a) Adriamycin (Thompson *et, al*.,1978)
b) Substances like etretinate, trans retinoic acid (Sasaki, 2004), and ethylene thiourea ETU (Maurício, 2007).
c) Vitamin A deficiency (Huang *et, al*, 2011)
d) The drugs most utilized are vitamin A derivatives and ETU

The utilization of rats enables study of larger fetuses, which is an advantage (Kluth, 2010). Qui *et, al,* (2002) studied the potential of ETU in inducing anorectal anomalies. The data obtained indicated that the ideal dose for inducing anorectal anomalies was 125 mg/kg. The anomalies most often found were rectourethral fistula in males and rectocloacal fistula in females (Kluth, 2010). ETU might cause disturbances in the mechanism for the action of the "sonic hedgehog" signaling molecule. Thompson *et,al*,(1978)Used Adriamycin to induce various anomalies in high percentages in rat fetuses. Observed were esophageal atresias with tracheoesophageal fistulas and ARMs amongst others. Some researchers studied environmental hazards and found associations between ARM and maternal alcohol intake (Yuan *et,al,*1995), tobacco smoke, and caffeine (Miller *et. al*,2009), benzodiazepine lorazepam Bonnot *et. al,*2001, paternal exposure to occupational hazards (Stoll *el.al,* 1997), and folic acid supplementation (Myers *et, al,* 2001). In addition to these factors, increased risks were found after in vitro fertilization (Reefhuis *et, al.,*2008). However, strong evidence is still scarce as most potential risk factors were found in only one study. Kolanut is a fruit of particular importance in the social life and religious customs of people in the tropics of West Africa (Adebayo *et. al,* 2001). It is highly valued for its perceived medicinal attributes which make it a highly desired product (Adebisi, 2004). *Cola nitida* offers several health benefits, such as: masticatory stimulant (Ibikunle and Ogbadoyi, 2011), it is a bronchodilatior and can be used to treat whooping cough and asthma (Esimone *et, al*, 2007). *Cola nitida* has been used to counteract hunger and thirst and also to control vomiting in the first trimester of pregnancy in women (Adisa *et, al,* 2010). Other therapeutic effects include the control of trichomoniasis, treatment of

morning sickness, migraine headache, and indigestion (Oluyole *et,al,* 2001). It can also be applied directly on the skin to cure wounds and inflammation (Ibu *et, al*, 1986), and used in cleaning the teeth and gums (Oluyole *et, al,* 2001). Other uses of Kola nut include the production of beverages, flavouring materials, alkaloids, caffeine, laxatives, heart stimulants and sedatives (Morton,1992). Adverse effects of kola nuts include aggravation of peptic ulcer symptoms due to its caffeine and its tannin content (Alaribe et, al, 2003), it is carcinogenic (Chukwu et, al, 2006), can mimic malaria-like symptoms(Benie and Theulant, 2003) and can cause cardiac arrhythmia as well as heart failure (Adisa *et, al,* 2010). The documented effects of *Cola nitida* on the reproductive system include anti-fertility effect on female rats but it has been shown to have no effect on semen quality (Infante-Rivard, 1993). Kola nuts contain large amounts of caffeine and threobromine and are therefore used as a stimulant (Jaiyeola, 2001). It is known that the substances called theophylline and caffeine facilitate in unwinding the muscles as well as widen the bronchioles in people suffering from asthma and bronchitis, hence kola nuts are often used to treat whooping cough and asthma (Blades, 2000) Caffeine contained by the kola nuts counteract hunger and thirst; control vomiting in pregnant women (Chukwu *et, al*., 2006). Traditionally, these nuts were chewed as a masticatory substance, to stimulate the flow of saliva (Leakey, 2001). The kola pod husk has been used in the manufacture of poultry feeds, snail feed (KOLA-T).10 to 15 percent dietary inclusions of Kolanut Pod Husk reduced feed cost while not sacrificing bird performance (Olubamiwa *et,al*, 2002). Kola nut extract is used in the food industry as a natural flavoring agent (Burdock, 1997). The extract also finds use as a natural herbal preparation for the treatment of mental and physical fatigue (Blumenthal, 1998). The nut itself is also exported worldwide for extraction and is used in the manufacture of methylxanthine-based pharmaceuticals (Blumenthal, 2000). Reports of caffeine content can vary between 1.5% and 3.8%, depending on the variety and treatment of the nut. Treatments include fresh (raw) nuts, cured nuts (6 months), sun-dried nuts (sun dried for 40 days), and, milled and stored nuts (sun-dried nuts milled and stored for 12 months) (Atawodi *et, al,* 2007). In Europe, Kolanuts were once used to treat migraines, neuralgia, nausea, and diarrhea (Ratsch,1998). Kola preparations are also used today to treat physical and mental exhaustion (Russel,1995). Kola nut is used in manufacturing of beverages such as Coca Cola and pepsi Cola (Javies, 2002).Several congenital syndromes have been associated with malformations affecting the development of anus and rectum, including the VACTERL syndrome, which represents a non-random association of vertebral, anorectal, cardiac, trachea-esophageal, renal, and limb anomalies includes another important cohort of patients with anorectal malformations (Jensen, 1998).

5. Materials and Methods

Plant material

Seeds of *Cola Nitida* were procured from a market in Bodija, Ibadan, Nigeria and were identified and authenticated by Mr F. Akinwumi of Herbarium section at Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria, where a voucher specimen was deposited. Phytochemical screening of the seeds was carried out at the Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria.

Preparation of the aqueous extract

The fruits were chopped to small pieces and dried in room air. The dried samples were grinded into powder, stored in polythene bags and placed at room temperature until they were ready to be used. Aqueous extraction of the sample was carried out by macerating 1000g portion of the powdered seeds with 5 liters of distilled water for 24hours, this was boiled for 15minute, allowed to cool down and filtered. The extract was evaporated to dryness at temperature of 40-45^{0}C[28].

Animal material

Twelve, adult female rats were used for the research. Animals were purchased from the common central animal house, University of Ibadan. They were housed in well ventilated cages and were allowed free access to feed and water *ad libitum,* following an approval from the University of Ibadan Ethical Committee on the use of animal for experiment.

The rats were divided into four groups of three animals as follows:
GROUP 1 (Control): Rats to receive distilled water orally for 21 days
GROUP E1: Rats to receive 200mg/kg of aqueous extract of *Cola nitida* orally throughout the period of pregnancy.
GROUP E2: Rats to receive 400mg/kg of aqueous extract of *Cola nitida* throughout the period of pregnancy
GROUP E3: Rats to receive 600mg/kg aqueous extract of *Cola nitida* throughout the period of pregnancy
Two adults male Wistar rats were grouped together with three adults female Wistar rats, the grouping was maintained for two estrous cycles to have passed. The rats were tested for pregnancy using the vaginal lavage and microscopic examination for the presence of spermatozoa. This day was considered as day zero (D0) of gestation.

All the rats were allowed to carry the pregnancy to term before they were delivered, the resulting dams were weighed and physically examined for presence of anorectal malformations and other observable associated anomalies. The dams were sacrificed by cervical dislocation, their anorectums dissected out and fixed with 10% formal saline for histologic analysis.

Analyses and Tests

The following analyses and test were performed on the pups:

Phytochemical studies – Phytochemical analyses of the aqueous extract of the seed of *Cola nitida* was carried out for anthraquinone, terpenoids, flavonoids, saponins, tannins, alkaloids, cardiac glycosides and caffeine.

Physical Examination – The pups were weighed using a digital weighing scale **(**American weigh Scale Gem-20**)** and they were examined to observe the presence of anomalies of the anorectum, and other associated anomalies like tail agenesis and limb defects in the experimental groups.

Haematological and Serobiochemical parameters of pups

Blood samples were collected from 5 pups from each group into ethylene diamine tetra acetic (EDTA) acid treated sample bottles for the determination of serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) using standard procedures. Blood for hematological parameters were obtained from the retro-ocular plexus of the pups using heparinized capillary tubes. The serum chemistry was carried out at the Veterinary Pathology Laboratory of University of Ibadan. five pups were sacrificed from each group and their livers were harvested for histological analysis with H & E stain. The variation in the different groups was compared using the control as reference.

Histology – The anus, Liver and the distal gastrointestinal tract were fixed in 10% formalin, embedded in paraffin and sectioned at 5µ using an automated microtome. The slides of the tissue sections were prepared and stained with hematoxylin and eosin.

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM). Data were analyzed using one-way ANOVA followed by Dunnette test. Values were considered statistically significant at $p<0.05$. All the data were processed with Graph Pad Prism Software Version 5.00.

6. Results

Animals

Gross morphology: A total number of 101 pups were delivered in both control and experimental groups with mean number of litters of 11.0±0.58 in the control group, 9.33±0.88 in experimental group E1, 8.33±0.33 and 5.00±0.58 in groups E2 and E3 respectively. The weight of the pups in group E3 is however significantly reduced in comparison with the weight of the pups in the control group (Table 2). None of the pups in both control and experimental groups had anorectal malformations or any other associated congenital anomalies.

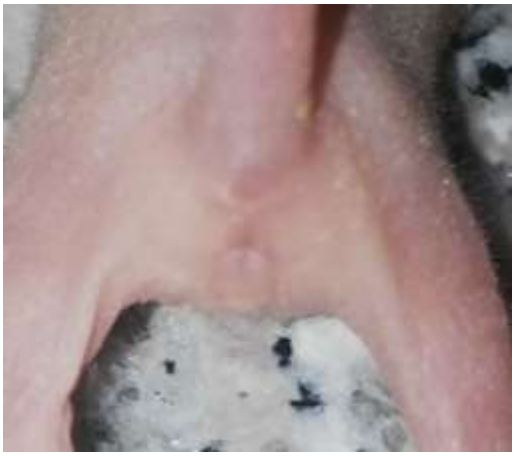
Pups of experimental groups were also observed to have decreased weight, especially pups of group E3 (600mg/kg) in comparison with the rats in the control group (Figure 1). There were no notable changes in behavior or clinical signs in the pups of both control and in treated dams.

Table 1: Phytochemical constituents of aqueous extract of *Cola nitida*

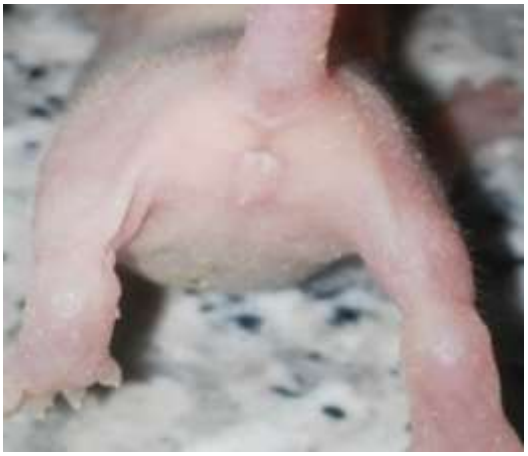
Phytochemicals	*Cola nitida*
Anthraquinone	+
Terpenoids	+
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+
Cardiac glycosides	+
Caffeine	+

+ = Present - =Absent

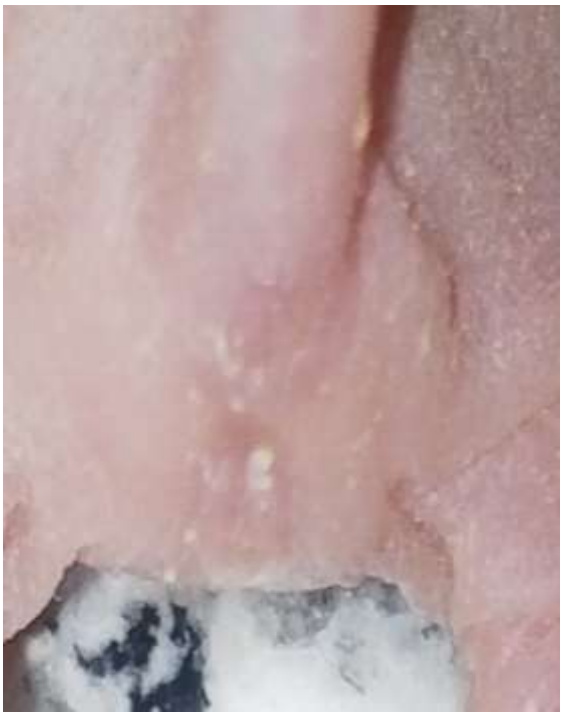
Physical observation



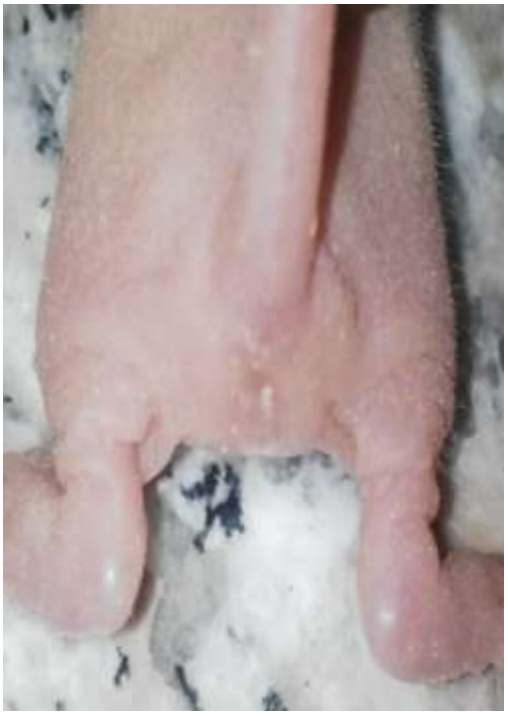
a. *Group 1 (Control)*



b. *Group 2 (200mg/kg)*



c. *Group 3 (400mg/kg)*



d*.Group 4 (600mg/kg)*

Fig. 1 a,b,c,d : *Effect of Cola nitida on the animals(physical examination)*

Table 2: Mean number of Litters per group

Parameter	Control	200mg/kg	400mg/kg	600mg/kg
Mean	11.00± 0.58	9.33±0.88	8.33±0.33	5.00±0.58*

*All the data expressed as Mean ± Standard Error of Mean (SEM) * p<0.05*

Table 3 :Effect of aqueous extract of *Cola nitida* on haematological and serobiochemical parameters of pups

Parameter	Control (n=5)	200mg/kg (n=5)	400mg/kg (n=5)	600mg/kg (n=5)
PCV%	33.00±2.59	26.80±3.33	37.60±1.72	33.00±2.47
Hb (g/dl)	10.16±0.62	12.00±0.61	9.28±1.03	11.30±0.79
RBC(×10 ^6)	5.72±0.44	4.88±0.59	6.14±0.30	5.27±0.52
Platelets(10^5)	0..36± 0.21	0.57±0.97	0.66±0.29*	1.13±0.53*
Neutrophil (%)	28.20±1.83	32.40±1.12	31.80±1.11	30.20±1.24
Lymphocytes (%)	69.20±0.97	64.80±2.13	62.80±1.02	61.80±1.83*
Eosinophil (%)	1.60±0.40	2.60±0.25	2.00±0.45	3.60±0.51*
Monocyte (%)	1.20±0.20	3.40±0.75*	3.40±0.50*	4.20±0.37*
AST (u/l)	32.80±1.39	34.60±2.32	37.80±2.71	39.20.±1.88
ALT (u/l)	24.40±1.20	22.60±1.08	21.40±1.94	16.80±0.97*
ALP (u/l)	101.8±0.73	128.20±3.26*	105.4±3.02	116±6.03
WBC (10^3/ul)	2.31 ±0.19	3.04 ±0.25	4.59 ±0.19*	6.01 ±0.29*

(*: Significant : p<0.05). All the data expressed as Mean ± Standard Error of Mean (SEM)

Histological assessment. **Fig. 3**

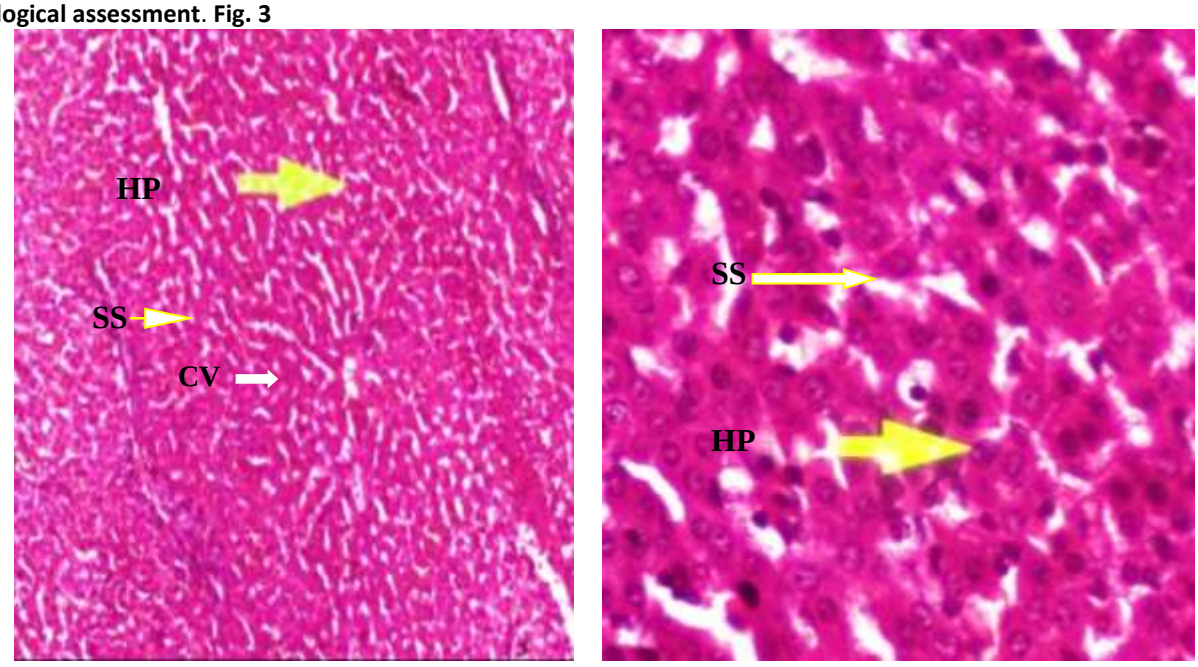
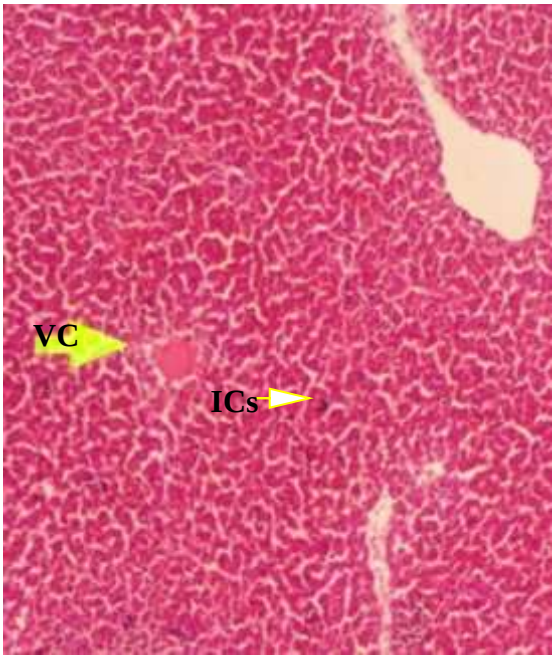


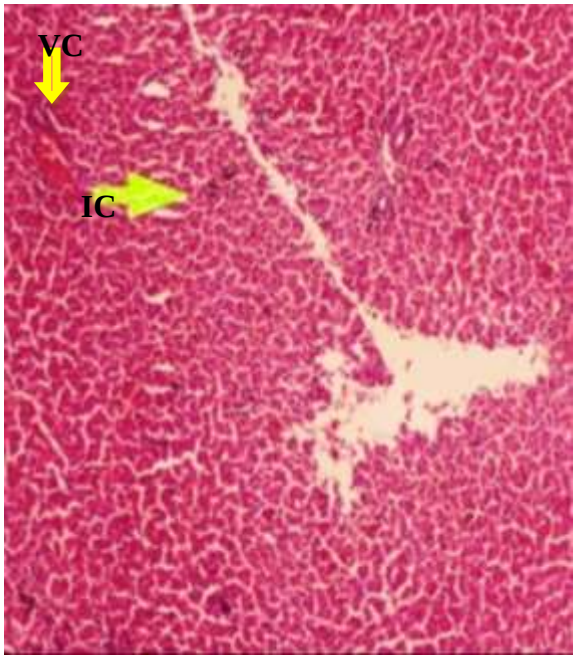
***Plate** 1. **A.** Control H & E x100* ***B**. Control H & E x 400*

Normal Architecture of Liver



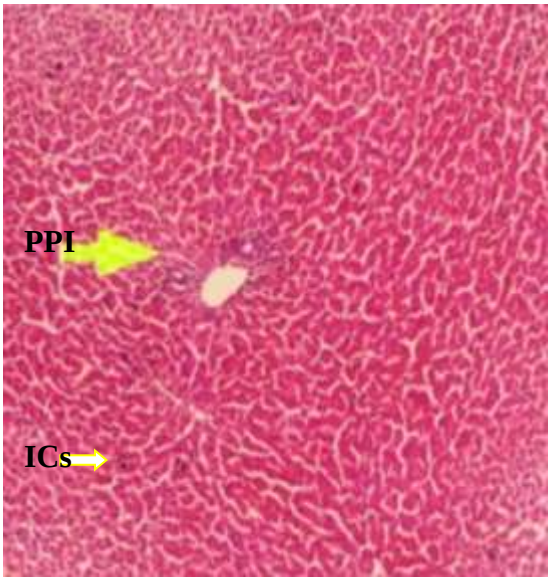
C. 200mg/kg H & E x100

Normal appearance of hepatocytes

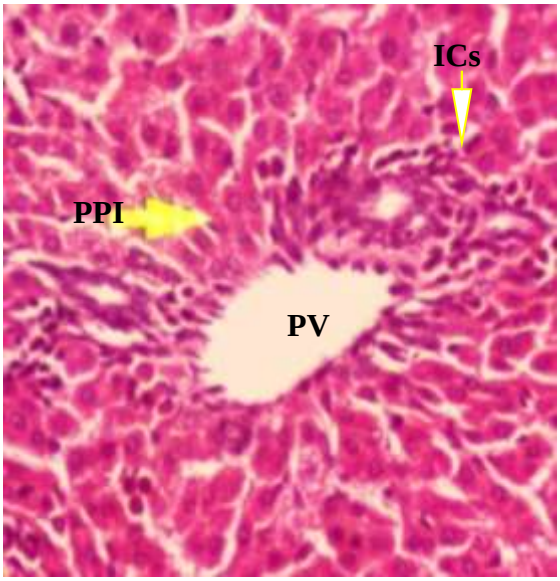


D. 400mg/kg H & E x100

Plate 2. *Photomicrograph of the liver showing the effect of aqueous extract of Cola nitida*.



& E x100



E.600mg/kg H

F.600mg/kg H & E x 400

Plate 3. *Photomicrograph of the liver showing the effect of aqueous extract of Cola nitida:* ***E .***600mg/kg H & E x100 ***F.*** *600mg/kg H & E x 400*

Inflammatory cells (ICs)
Periportal inflammation (PPI)
Vascular congestion (VC)
Portal Vein (PV)
Central vein (CV)

7. Discussion

Cola nitida seeds extract causes damage of vital organs exemplified by decrease in weight of litters. Oral administration of *Cola nitida* at high doses to female pregnant rats was observed to have induced a progressive decrease in the number of litters per rats. The decrease in number of pups per rat might be attributed to the effect of the extract on ova production during oogenesis (Agbia *and Ugwu, 2012)*. Ajarem and Ahmad (1994) had earlier reported that Enzyme Linked Immunosorbent Assay (ELISA) method showed that dose concentration of 6mg/kg and 10mg/kg of aqueous extract of *Cola nitida* may cause a significant reduction in the serum level of follicle stimulating hormone (FSH); although serum level of luteinizing hormone (LH) was significantly reduced at all the dose concentrations. Consequently, this may alter the oestrous cycle of the animals (Sanin *et,al*,2001). The weight of the ovary and uterus was also statistically reduced only at dose concentration of 100mg/kg body. Thus, indicating the anti-fertility and anti-gonadotropic effect of aqueous *Cola nitida*. Administration of *Cola nitida* to female pregnant rats during the period of pregnancy produced significant decrease in weight of pups especially in pups of pregnant rats that received 600mg/kg. These changes may be attributed to deficiency of nutritional supply from dam to fetuses. Fetal nutrition and intrauterine growth restriction have a lot to do with functional placenta (Metzenbauer,2002), reduced placenta size, volume and weight (Ayoola *et, al,*2008). Whereas increase in placenta weight during pregnancy has been well established to initiate corresponding fetal weight gain (Ajarem,1990), increased maternal thyroxine level has also been shown to stimulate placenta growth (Spencer and Robinson,1993). Therefore, the reduction in weights of litter, of all rats treated with the *Cola nitida* extract might be as a result of the decrease in placenta weight precipitated by the decrease in T3 level. The reduction in weights of litters might also be due to general vasoconstriction of placental vessels leading to a reduction in utero-placental blood flow during pregnancy. Caffeine is a known vasoconstrictor and a major constituent of Kola nut. Pups of pregnant rats given aqueous extract of 200mg/kg, 400mg/kg and 600mg/kg *Cola nitida* presented with no anorectal malformations, tail agenesis or short tail and there were no urological structural alterations like unilateral kidney agenesis, hypoplasia of the kidneys and horse-shoe kidneys following gross examination for external and internal malformations macroscopically. This suggests that *Cola nitida* may interfere with fetal growth during pregnancy because it contains a potent vasoconstrictor, it does not have a teratogenic effect on the animals at the doses administered. The hematological study has revealed that the extract caused significant increase in WBC which may be as a result of the body building the immunity of the fetus confirming the immunostimulatory effect of the extract. The extract also caused significant increase in platelets value in the group of 400mg/kg and 600mg/kg. The extract increased monocyte level which is probably indicative of the enhancement in the phagocytic function of the blood. Also, the extract increased eosinophil levels which might be indicative of the anti-allergic and anti-parasitic infectious responses. The pups of the experimental rat given showed a decrease in the activity of ALT which might be attributed to the damage and injury in the liver resulting in an inability of the hepatocytes to synthesize the enzyme. The extract also caused non-significant increase in the activity of AST which can be attributed to the damage in the liver and heart. The histology of the liver showed Vascular congestion with interstitial edema, focal areas of inflammation and periportal inflammation. There are also widening sinusoids of the liver, suggestive of portal hypertension in all the experimental groups. This result demonstrated the embryotoxicity of the extract.

8. Conclusion

The consumption of *Cola nitida* early in pregnancy caused a significant antifertility effect and intrauterine growth restriction in the pups of the rats suggesting that it may have an embryotoxic effect and cause infertility in rats. However, its teratogenic effect can be studied further as *Cola nitida* seeds are consumed excessively by humans in west Africa.

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Conflict of Interest: The author declares no conflict of interest.

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