OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2017.73.77

Research Article
Effect of Aqueous Extract of *Kola nitida* on the Development of Biliary Atresia in Pups of Wistar Rats

[1]Ibitoye Babatunde Oluwaseun and [2]Ogundoyin Olakayode Olaolu

[1]Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria
[2]Department of Surgery, College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract

Background and Objective: Biliary atresia (BA) is a congenital malformation characterised by a progressive fibrosing obliteration of the extra hepatic biliary system manifesting during the 1st week of life. The objective of this study was to investigate the teratogenic properties of aqueous extract of *kola nitida* as a cause of biliary atresia in wistar rats. **Materials and Methods:** Twenty adult female wistar rats were randomly distributed into 4 groups with weight ranging from 180-200 g. The female rats were grouped with 2 male rats for the purpose of mating. Following confirmation of pregnancy, variable oral doses of aqueous kola nut extract were given from 1 day of pregnancy till delivery of the pups. Data was analyzed by one-way analysis of variance (ANOVA) using SPSS. **Results:** The liver to body weight ratios of the pups were significantly reduced in treated groups compared to the control. Also, the average number of litters per rat was significantly reduced in treated groups compared to the control group. The liver enzymes, bilirubin (conjugated and total) and the histology of the livers were essentially normal. There was no other abnormality observed. No case of biliary atresia or other congenital abnormality was recorded following the administration of aqueous extract of *kola nitida* to the mother rat during pregnancy. **Conclusion:** The safety of the extract in pregnancy cannot be assured due to observation of reduced pup number and liver to body weight ratio in association with increased dosage.

Key words: *Kola nitida*, biliary atresia, bilirubin, congenital malformation, teratogenic properties

Citation: Ibitoye Babatunde Oluwaseun and Ogundoyin Olakayode Olaolu, 2017. Effect of aqueous extract of *Kola nitida* on the development of biliary atresia in pups of wistar rats. Pharmacologia, 8: 73-77.

Corresponding Author: Ibitoye Babatunde Oluwaseun, Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria
Tel: +2348030819267

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biliary atresia is a congenital malformation in which there is obliteration of intrahepatic and extrahepatic bile ducts leading to progressive liver injury[1]. It is the third most common cause of neonatal cholestatic jaundice and is the most common indication for liver transplantation in children[2]. The incidence of biliary atresia varies approximately from 1 in 8,000 to 1 in 15,000 live births. Theoretical considerations of the pathogenesis are based largely on epidemiological and clinical features, reported predisposing genetic factors and the pace of disease progression[3]. The association between biliary atresia and occlusion of portal vein and hepatic artery suggested that an intrauterine ischaemic event may exert some influence upon the development of bile ducts and may play a role in the pathogenesis of biliary atresia[4,5]. The consequence is the development of obstructive jaundice[6].

Cola nitida (Kola nut) is a seed of a tropical tree belonging to member of the Sterculiaceae family which has dose-dependent antifertility effect[7,8]. Caffeine is the main constituent of *Cola nitida* that is majorly responsible for its physiological activities, other constituent were methylxanthines, theobromine, flavonoids, anthocyanins and tannins[9-12]. Consumption of caffeine has also been implicated as a risk factor for spontaneous abortions[13-15].

Biliary atresia is a disease of great burden, whose etiology is largely unknown, kola nut and its major constituent is widely consumed and has been found to affect smooth muscle (including those in blood vessels) which could cause vasoconstriction in utero and consequently atresia of the biliary tree and also it has been linked to other teratogenic effects, which justifies this study.

The objective of present study was to investigate the effect of aqueous extract of *kola nitida* on the development of biliary atresia in pups of wistar rats.

MATERIALS AND METHODS

Twenty adult female wistar rats weighing between 140-200 g were obtained from the Animal House Veterinary Department, University of Ibadan, Oyo State, Nigeria and the experimental work was carried out in the Animal House of Human Anatomy Department of the same university around April, 2015 and were randomly distributed into 4 groups of 5 rats per group following an approval from the University of Ibadan Ethical Committee on the use of animals for experiments. The groups were, group A: The control which received only distilled water, group B: Which received 200 mg kg^{-1} of aqueous extract of kola nut each day, group C: Which received 400 mg kg^{-1} of aqueous extract of kola nut each day and group D: Which received 600 mg kg^{-1} of aqueous extract of kola nut each day throughout the pregnancy. The rats were allowed to acclimatize to the animal house condition for 2 weeks with the animals receiving commercial feed and water *ad libitum*, under constant environmental conditions of temperature and humidity. Two adult male wistar rats were grouped together with 5 adult female wistar rats, the grouping was maintained for 10 days to allow for at least 2 oestrous cycle to have passed[16]. Evidence of fertilization was observed with microscopic examination of vaginal smear for presence of spermatozoa. The animals whose results come out positive were considered to be potentially fertilized. This was considered to be day zero of gestation. The rats were administered aqueous extract of kola nut orally for a period of 20 days by means of an oral gavage.

Animal sacrifice and sample collection: Blood samples were obtained from the animals via cardiac puncture and serum was obtained from the blood of pups in both the experimental and control groups for total and conjugated bilirubin, serum protein and albumin and level of liver enzymes which were measured using immunoassay method. The following were assayed, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), total and conjugated bilirubin with serum protein and albumin of pups of both control and treated wistar rats.

Tissue preparation for light microscopy: The liver of the pups and extra-hepatic biliary tree were harvested and immersed in 10% formalin, dehydrated and cleared, few days after delivery. The sections were stained with Hematoxylin and Eosin, examined and photomicrographed.

Statistical analysis: Data obtained were presented as means±standard error of mean. Results were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical package of social science version 16 $p<0.05$ was considered statistically significant.

RESULTS

The total numbers of pregnant rats were 14 and they delivered a total of 105 pups. There was no mortality or still birth. More pups were produced by rats in Group A (Control group) and Group B with an average of 8 pups per rat while rats in Group C and Group D produced less pup at an average

Table 1: Effect of *Cola nitida* extract on average number of litters per rat in each group

Groups	Number of pregnant rat	Number of pups	Mean number of pups
A (Control)	3	25	8.33±0.57
B (200 mg kg^{-1})	4	33	7.75±0.96
C (400 mg kg^{-1})	4	28	6.00±1.41
D (600 mg kg^{-1})	3	19	5.67±1.52

Mean±SEM, A: Control, B: 200 mg kg^{-1} of AECON only, C: 400 mg kg^{-1} of AECON, D: 600 mg kg^{-1} of AECON

Table 2: Effect of *Cola nitida* extract on weight of liver per weight of the pup

Groups	Organ to weight ratio
A	0.033±0.0038
B	0.029±0.0033
C	0.025±0.0039
D	0.023±0.0042

Mean±Standard Error of Mean, A: Control, B: 200 mg kg^{-1} of AECON only, C: 400 mg kg^{-1} of AECON, D: 600 mg kg^{-1} of AECON

Table 3: Biochemical parameters of aqueous extract of *Cola nitida* on the pups of wistar rats

	Groups			
Parameters	I	II	III	IV
ALT (U L^{-1})	27.90±2.80	25.60±2.60	24.40±1.50	22.80±1.80
AST (U L^{-1})	8.50±0.60	7.60±0.20	6.10±1.10	5.60±0.40
ALP (U L^{-1})	5.40±0.80	5.00±0.60	4.80±0.40	4.60±0.20
TSP (g dL^{-1})	0.80±0.22	0.76±0.60	0.70±0.40	0.67±0.50
TBIL (g dL^{-1})	0.65±0.07	0.61±0.04	0.58±0.02	0.54±0.05
CB (g dL^{-1})	0.24±0.02	0.18±0.04	0.14±0.03	0.12±0.01

AST: Aspartate transferase, ALT: Alanine transferase, ALP: Alkaline phosphatase, TBIL: Total bilirubin, CB: Conjugated bilirubin, TSP: Total serum protein Mean±SEM
The difference in the values of biochemical parameter between control and treated groups was not statistically significant $p<0.05$

of 6 pups per rat, this is summerize in the Table 1. There was a dose related reduction in the number of pups per rat (Table 1). The mean maternal weight of the rats were 136 g.

Subjectively, it was observed that rats receiving higher doses of the extract were more active and aggressive compared to the control group.

Gross examination: Examination of the livers, extra-hepatic biliary trees and the vasculature of the liver of the pups did not reveal any gross abnormalities and there were no other associated congenital abnormalities found in the animals. However, there was a reduction in the weight of the livers of the pups per body weight and this was observed to be more with increasing dose of the aqueous kola nut extract given (Table 2).

Effect of aqueous extract of *Cola nitida* on the histological profiles of the liver: In Fig. 1(a-d) and d liver and the biliary system shows normal histological architecture. No evidence of histological alteration or damage or histological feature of BA was observed in any of the slide. There was normal appearance of hepatocytes.

Effect of aqueous extract of *cola nitida* on biochemical parameters of pups: The difference in the values of biochemical parameter, which were liver enzyme and bilirubin in its various form, between control and treated groups was not statistically significant as shown in Table 3.

DISCUSSION

There was a significant reduction in the average number of pups produced per mother rat in each group with increasing dose of the extract given, also observed was the increased average time between introduction of male to the group and conception in groups receiving higher doses of the extract. This agrees with the findings of Adisa *et al.*[8], who described dose dependent anti-fertility effect of aqueous extract of *Cola nitida* on female wistar rat and other researchers who found out that caffeine consumption has also been implicated as a risk factor for spontaneous abortions and delayed conception[8,13-15,17]. They reported that certain concentrations of aqueous extract of *Cola nitida* significantly reduced the serum level of follicle stimulating hormone, although serum level of luteinizing hormone was significantly reduced at all the dose concentrations. This might account for reduced fertility observed in this study.

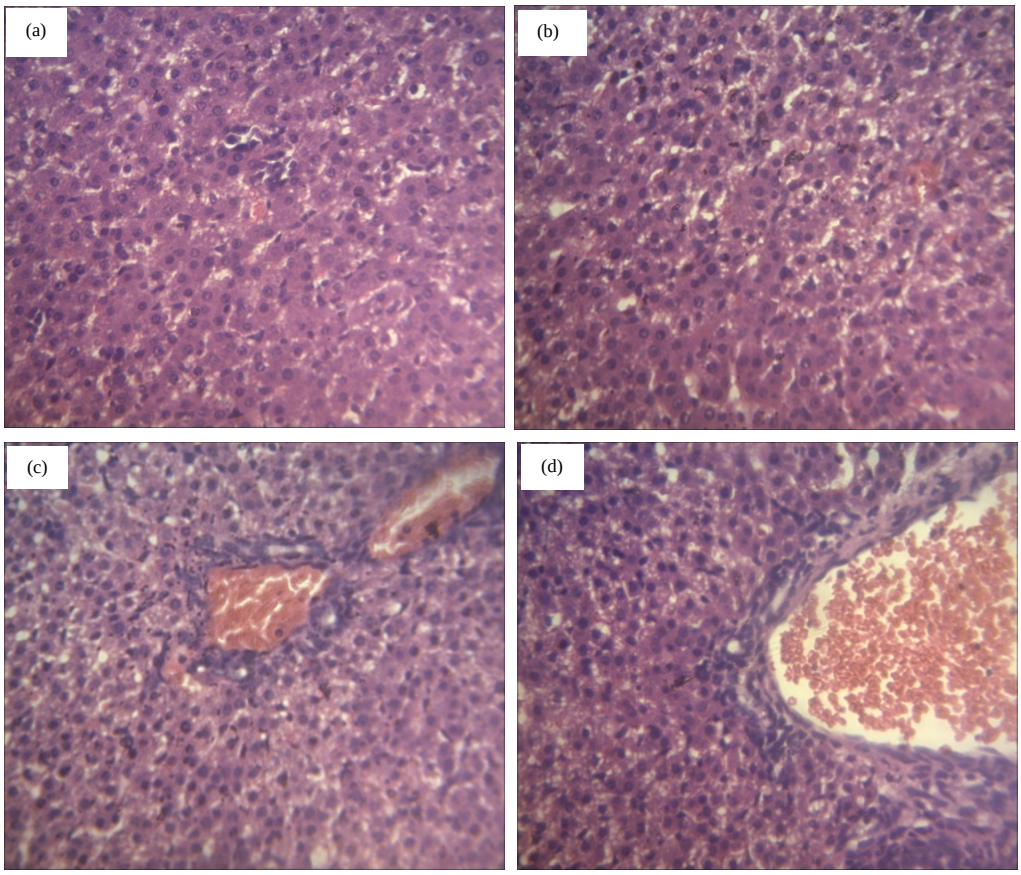

Fig. 1(a-d): Micrograph images (a) Pup's liver in the control group H and E, X 400. Normal liver architecture. Normal appearance of hepatocytes, (b) Pup's liver in 200 mg kg^{-1} of AECON-treated rat group H and E x400, (c) Pup's liver in 400 mg kg^{-1} of AECON-treated group H and E x400, (d) Pup's liver in 600 mg kg^{-1}-treated rat group H and E x400

The ratio of weight of the pup's liver to body weight of the pups was noticed to be reduced with the consumption of aqueous extract of kola nut. Abnormal contraction of vascular smooth muscles leading to vasoconstriction is a major cause of certain diseases, such as hypertension and vasospasm in coronary and cerebral arteries[18]. Caffeine which is one of the major constituents of kola nut has being observed to cause smooth muscle contraction in some mammals[19,20,13-15].

This could have caused contraction of smooth muscles in the wall of blood vessels and subsequent vasoconstriction may reduce perfusion of the targeted organs and tissues leading to growth restriction.

The association between biliary atresia and occlusion of portal vein and hepatic artery suggested that an intrauterine ischaemic event may exert some influence upon the development of bile ducts and may play a role in the pathogenesis of biliary atresia[4,5]. A vascular ischaemic aetiology for biliary atresia has been proposed based on direct experimental evidence[4]. The progressive inflammatory and sclerotic reactions result in the eventual obliteration of the biliary tree. The consequence is the development of obstructive jaundice, indicated by direct hyperbilirubinemia and passage of alcoholic stools[6]. However, the histological and biochemical findings in this study were not in keeping with the normal pictures in biliary atresia (Fig. 1a-d). There were no significant variations in the serum levels of bilirubin, glutamic oxaloacetic aminotransferase (SGOT), glutamic pyruvic transaminase (SGPT), alkaline phosphatase, (ALP), total protein and albumin between the control and the treated groups. Elevation of these biochemical markers and substrates were pointers to liver damage and obstruction to bile flow.

Consumption of caffeine during pregnancy has been suggested to cause birth defects in human offspring similar to that seen in animal studies but there is a dearth of information on its teratogenic effect[21]. In the present study, with gross physical examination, no congenital abnormalities were observed and there was no spontaneous abortion or mortality during the experiment.

CONCLUSION

The aetiology of biliary atresia was still largely unknown despite its burden on the health of affected babies, the government and the available resources to treat the anomaly. Understanding its aetiology may be of immense help towards reducing this heavy burden.

It was noteworthy to observe that aqueous extract of kola nut does not have teratogenic effect but may interfere with fertility when consumed excessively.

No case of biliary atresia or other congenital abnormality was recorded by giving the mother rat aqueous kola nut extract during pregnancy in the research. However, adequate knowledge of the detailed pharmacology of the extract and the differences in genetic predisposition to the development of the disease may be a key factor to determine who will have the disease. Observation of reduced pup number in association with increased dosage is a pointer to exercising caution in the consumption of kola nut during pregnancy.

SIGNIFICANCE STATEMENT

This study discovered that consumption of kola nut and its derivatives in beverages during pregnancy do not cause biliary atresia though cause some degree of other hepatic pathology in the offspring and negative effect on the reproductive potential of the mother.

REFERENCES

1. Pall, H. and M.M. Jonas, 2005. Cholestatic disease an issue of clinics in liver disease. J. Gastroenterol., 21: 257-259.
2. Balistreri, W.F., 1985. Neonetal cholestasis-medical progress. J. Paediatrics, 106: 171-174.
3. Wong, K.K.Y., K.L. Chan, H. Saing, S.T. Fan and P.K.H. Tam, 2003. The management of biliary atresia in the era of liver transplantation. J. Paediatrics, 8: 322-326.
4. Klippel, C.H., 1972. A new theory of biliary atresia. J. Pediatric Surg., 7: 651-654.
5. Ho, C.W., K. Shioda, K. Shirasaki, S. Takahashi, S. Tokimatsu and K. Maeda, 1993. The pathogenesis of biliary atresia: A morphological study of the hepatobiliary system and the hepatic artery. J. Pediatric Gastroenterol. Nutr., 16: 53-60.
6. Narkewicz, M.R., 2001. Biliary atresia: An update on our understanding of the disorder. Curr. Opin. Pediatrics, 13: 435-440.
7. Keay, R.W.J., C.F.A. Onochie and D.D. Stanfield, 1964. Nigerian Trees. Vol. 2, Department of Forest Research, Nigeria, pp: 378-398.
8. Adisa, W.A., H.O. Otamere, C.U. Osifo, O.B. Idonije and E.O. Nwoke, 2010. Effects of aqueous extract of Kola nut (Cola Nitida Rubra) on reproductive hormones in rats. Nig. J. Physiol. Sci., 25: 121-123.
9. Van Eijnatten, C.L.M., 1969. Kola, its botany and cultivation. West Afr. Sci., 210: 224-227.
10. Harborne, J.B., H. Baxter and G.P. Moss, 1999. Theobromine. In: Phytochemical Dictionary, Harbone, J.B., H. Baxter and G.P. Moss (Eds.). 2nd Edn., Taylor and Francis, Philadelphia, PA., pp: 123.
11. Burdock, G.A., 2005. Kola Nut (Cola Nut). In: Fenaroli's Handbook of Flavor Ingredients, Burdock, G.A. (Ed.). 5th Edn., CRC Press, Boca Raton, pp: 993-994.
12. Umoren, E.B., E.E. Osim and P.B. Udoh, 2009. The comparative effects of chronic consumption of kola nut (*Cola nitida*) and caffeine diets on locomotor behaviour and body weights in mice. Nig. J. Physiol. Sci., 24: 73-78.
13. Srisuphan, W. and M.B. Bracken, 1986. Caffeine consumption during pregnancy and association with late spontaneous abortion. Am. J. Obstetr. Gynecol., 154: 14-20.
14. Fenster, L., B. Eskenazi, G.C. Windham and S.H. Swan, 1991. Caffeine consumption during pregnancy and spontaneous abortion. Epidemiology, 2: 168-174.
15. Infante-Rivard, C., A. Fernandez, R. Gauthier, M. David and G.E. Rivard, 1993. Fetal loss associated with caffeine intake before and during pregnancy. Am. J. Med., 70: 2940-2943.
16. Marcondes, F.K., F.J. Bianchi and A.P. Tanno, 2002. Determination of the estrous cycle phases of rats: Some helpful considerations. Braz. J. Biol., 62: 609-614.
17. Jensen, T.K., T.B. Henriksen, N.H.I. Hjollund, T. Scheike and H. Kolstad *et al*., 1998. Caffeine intake and fecundability: A follow-up study among 430 Danish couples planning their first pregnancy. Reprod. Toxicol., 12: 289-295.
18. Yojikawano, T.Y. and K.N. Kozo, 2002. Pathogenesis and type of atresia. J. Med. Sci., 65: 1-8.
19. Ito, Y. and H. Kuriyama, 1971. Caffeine and excitation-contraction coupling in the guinea pig taenia coli. J. Gen. Pkytiol., 57: 448-463.
20. Siegman, M.J. and A.R. Gordon, 1972. Potentiation of contraction: Effects of calcium and caffeine on active state. Am. J. Physiol. Legacy Content, 222: 1587-1593.
21. Chukwu, L.O., W.O. Odiete and L.S. Briggs, 2006. Basal metabolic regulatory responses and rhythmic activity of mammalian heart to aqueous kola nut extracts. Afr. J. Biotechnol., 5: 484-486.