Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 4 April 2022

Research Article

# Clinico-Pathological Manifestations of Aqueous Extract of Kolanut Nitida on Four-Month-Old Puppies

**Barde Israel Joshua[1]*, Igboa Oziegbe Paul[2], Abubakar Sadiq Abubakar[2], Ishaku Leo Elisha[1], Leo Shedua Nyam[1], Shekaro Audu[1], Logyang Lot Emmanuel[1], Suleiman Isa[1], Ugbe Dominic Aondona[1] and Ngulukun Sati Samuel[1]**

[1]*Department of Diagnostic Veterinary Pathology, National Veterinary Research Institute, P.M.B. 01 Vom, Plateau, Nigeria*

[2]*Federal College of Animal Health and Production Technology, Vom, Plateau, Nigeria*

***Corresponding Author:** Barde Israel Joshua, Department of Diagnostic Veterinary Pathology, National Veterinary Research Institute, P.M.B. 01 Vom, Plateau, Nigeria.

**DOI:** 10.31080/ASVS.2022.04.0344

**Received:** February 21, 2022

**Published:** March 02, 2022



## Abstract

**Background:** This study investigated the clinical and pathological manifestations of kola nut on puppies. Kola nut which contains caffeine is used in some regions in Nigeria particularly the south-south to poison stray dogs.

**Materials and Methods:** Aqueous extracts of Kola nut nitida was administered to dogs in order to investigate the possible effects on some organs of the dogs. Four dogs were used in the treatment group whereby two doses of the extract (400 mg/Kg and 800 mg/Kg) were daily administered orally to the test animals for a period of 2 weeks.

**Results:** A dose related response was observed. Clinical signs observed include: excitement, polyuria, shivering, dull appearance, rough hair coat, emaciation/ body weight lost, drowsiness, tetanic convulsion, bloody diarrhea, aggressiveness, irritating mood, decreased pulse rate, decreased body temperature, decreased respiratory rate, sunken eye ball and mucoid diarrhea. Severity of gross lesions in the liver, kidney, Brain, lymph nodes and small Intestine of animals in the infected groups. The laboratory result showed that kola nut extract can be toxic to dogs following the laboratory results gotten from this study as haematology result showed that there is a difference in Red Blood cells, white blood cell, Monocyte, Pack cell volume, Neutrophils, Lymphocyte and Hemoglobin which shows hypochromic condition, while the histopathological lesions shows neuronal necrosis and pyknosis in the brain, gastric erosion with hemorrhage in intestine, necrosis of the renal tubular epithelial cells of the kidney, necrotic myocyte in the heart, thickening of the alveolar septa in the lungs and in the liver necrosis of the hepatocytes. Some of the biochemical parameters showed great statistical significance: AST, Urea, Total protein and Creatinine are high compared with the control group. These signify kidney and liver damage.

**Conclusion:** Despite the reported potentially beneficial effects of kola nut, its use as a medicinal plant should be with great caution because of the clinico-pathological manifestations seen in this study.

**Keywords:** Kola Nut; Dogs; Caffeine; Clinical Signs; Gross Lesions; Biochemical and Histopathology

## Introduction

Kola nut usually refers to the seeds of certain species of plant of the genus *Cola* that belongs to the family Malvaceae [1]. Cola species are evergreen trees native to tropical rainforests of Africa. The kola nut is a caffeine-containing nut with two species namely *Cola acuminata* and *Cola nitida* respectively [1]. *Cola acuminata* grows to approximately 20 metres in height and has long, ovoid leaves pointed at both the ends with a leathery texture. The trees have cream flowers with purplish-brown striations, and star-shaped fruit. Inside the fruit, about a dozen prismatic seeds develop in a white seed-shell. The nut's aroma is sweet and rose-like. The first taste is bitter, but it sweetens upon chewing. The nut can be boiled to extract caffeine. Kola nuts contain about 2-4% caffeine and theobromine, [1], as well as tannins, alkaloids, saponins, and flavonoids. The seeds are used as flavouring ingredients in beverages - the name 'cola', applied to various carbonated soft drinks originates [2].

Caffeine is a natural plant alkaloid found in more than 60 plant species and many beverage and food products, over the counter dietary supplements, and medications [3]. Caffeine is the common name for the chemical 1,3,7-trimethylxanthine, which is absorbed through the gastrointestinal tract and metabolized by the liver [3,4]. Caffeine is generally considered a safe compound because it has a high threshold of toxicity and is a gastric irritant with emetic properties [5]. Reports of toxicosis in the medical literature have been attributed to both accidental and intentional overdose [6-11]. The few case reports in dogs have been related to accidental ingestion or presumptive malicious poisoning [12-15]. Certain foods, which are completely safe for people and other animals, may cause problems if consumed by dogs and cats, due to metabolic differences in these animal species. Some products are likely to cause only mild digestive disorders, whereas others may result in severe toxicosis or even death. Most of the food-associated toxicosis cases are caused by ingestion of foods, because the owners are not aware of their toxicity. Furthermore, the mechanism of toxicity for some of them remains unknown, therefore they are still under investigation [16-19].

Cases of toxicosis are frequent admitted to veterinary clinics and most of them, according to the literature, during and holidays [17,19]. Most cases of food-associated toxicosis affect dogs compared to cats [16,20]. Due to their particularly curious nature and the tendency to investigate everything in their close environment, dogs are most often exposed to toxic substances and products, whereas cats are rarely affected, because they are attached to their unique dietary habits, which are often limited [16,19]. Cats represent only 11-20% of the recorded cases, a percentage three times smaller than that of canine cases [17].

Foods widely consumed by humans, such as chocolate, onions, garlic, grapes, xylitol, avocado, macadamia nuts and dough are potentially toxic to pets and should not be fed to them. The majority of methylxanthinetoxicoses have been reported in dogs and this is attributed to the canine preference for sweets [18,19]. All types of chocolate are considered to be toxic, however the severity of signs depends on the methylxanthine content of each product [17,20]. In particular, dark chocolate is the most toxic, milk chocolate is less toxic, whereas white chocolate must be consumed in large amounts in order to cause toxicosis [17-19]. The exact amounts of these food products which can result in toxicosis after ingestion have not been fully clarified and vary among studies. Other factors with a key role in causing food-associated toxicosis include body weight, general health condition, stomach content and the type of product that was consumed [17]. The mean lethal dose of caffeine and theobromine for dogs is 100-500 mg kg, which corresponds to four bars of dark chocolate. At the low dose of 20-40 mg kg mild clinical signs may occur (hyperactivity, vomiting), the dose of 40-50 mg kg may result in cardio toxic effects such as cardiac arrhythmias, at the dose of 60 mg kg seizures may be develop, whereas higher doses can be fatal [21-23].

Theobromine and caffeine are easily absorbed by the gastrointestinal tract, widely distributed throughout all tissues [17,24] and can cross the blood-brain barrier [16]. They are metabolised by the liver, in which they undergo enterohepatic circulation, and they are excreted mostly in the urine and in limited amounts in the faeces. It is worthy of note that methylxanthines may directly be transferred into milk and pose a high risk for suckling animals. The half-life of theobromine and caffeine in dogs is 17.5 and 4.5 hours respectively [17,18,24,25]. The fact that methylxanthine elimination rate in dogs is much slower than in any other animal species is the reason why dogs are so susceptible to this particular toxicosis [16,20]. Methylxanthines antagonise cellular adenosine receptors, resulting in severe stimulation of the central nervous system (CNS) and effects on the cardiovascular and respiratory system. Blockade of adenosine effect can induce a positive chronotropic and inotropic effect on the myocardium, vasoconstriction and to a small extent, diuresis. Methylxanthines also increase the intracellular content of calcium which results in increased contractility of the skeletal muscles, antagonise the benzodiazepine receptors, inhibit phosphodiesterase enzyme and increase systemic circulation levels of epinephrine and norepinephrine [17,20,21,24,26]. Methylxanthine overdose steadily increases the severity and duration of all these metabolic processes, leading to death by cardiorespiratory failure [16,19]. The first clinical signs of toxicosis usually emerge within 2-12 hours after ingestion and usually include salivation, vomiting, diarrhea, polydipsia and polyuria. Clinical signs develop quickly followed by neurological signs such as restlessness, muscle tremors, ataxia, hyperactivity, seizures, hyperthermia, tachypnea, cyanosis, tachycardia, arrhythmias and finally death [17,18,24-26].

Diagnosis is based on history, in combination with clinical signs [16,24]. Toxicosis due to organophosphates, mycotoxins, strychnine, nicotine, amphetamines, pseudoephedrine, antihistamines, antidepressants, or generally other CNS stimulants should be included in the differential diagnosis [26]. There is no specific anti-

dote for methylxanthine toxicosis [20]. Treatment is symptomatic, supportive and focused on decontamination [20,21,24]. Once the animal is admitted, the first step should be the induction of emesis, if indicated, or when this is not possible or contraindicated, gastric lavage should be performed, followed by the administration of adsorbent agents which prevent further gastrointestinal absorption of toxic substances [21,25]. Furthermore, aggressive intravenous fluid administration is also indicated, aiming in increasing the elimination rate of toxic substances [17]. In cases in which methylxanthine toxicosis has resulted in seizure activity, diazepam as a general measure for seizure control may prove ineffective, because of benzodiazepine receptor antagonism by methylxanthines. Alternatively, phenobarbital, propanol, and inhalational anaesthesia have been recommended in this order for the management of seizure activity. In cases of cardiac arrhythmias, these are managed according to the type of arrhythmia. Finally, gastro protective agents are administered if there is refractory vomiting [25]. It is generally good but can become guarded in cases admitted with severe neurological signs [20,24].

This study investigated the clinical and pathological manifestations of kola nut on puppies so as to check the kola nut containing caffeine and theobromine because kola nut is mostly used in some regions in Nigeria particularly south-south to poison stray dogs which has variety of pharmacological effects with respect to cognitive performance (alertness), motor activity, which also stimulate renal diuresis (polyuria), vomiting, tachycardia, cardiac muscle stimulation, diarrhea, cyanosis, ataxia, arrhythmias and finally death, which tends to affect the production of dogs in the society [17,18,24-26]. Foods widely consumed by humans, such as chocolate, onions, garlic, grapes, xylitol, avocado, macadamia nuts and dough are potentially toxic to pets, dogs should not be exposed to food substances containing caffeine because of their low rate of metabolizing caffeine, as it possess risk factors on their health. And should not be fed to them. These cases usually require immediate general decontamination measures, mainly induction of emesis when this is deemed safe as well as specific treatment. Appropriate education of companion animal owners for the risks of food associated toxicoses contributes to the prevention of such incidents, as well as their immediate and proper management [27]. The aim of this study is to evaluate clinico-pathological manifestations of kola nut on four months old puppies.

## Materials and Methods

### Study area

The research was carried out in the Veterinary Pathology Division of National Veterinary Research Institute Vom, Jos South Local Government Area Plateau State Nigeria.

### Sample collection

The kola nut seed cola nitida used in this study were purchased from Ewu market in Esan Central Local Government area of Edo State, Nigeria.

### Extraction of sample

Samples were extracted using the method described by [28]. 8kg of Cola nitida were pulverized using mortal and pistol the pulverized product were obtained and was extracted with using hot water at 96°c by soaking the sample for 24 hours. The powdery product were obtained, from which aqueous extract of 8 mg/kg body weight were prepared.

### Phytochemical analysis

Phytochemical analysis of kola nuts nitida were carried out in the Biochemistry Laboratory of Federal College of Animal Health and Production Technology, Vom.

### Procedure

- *Saponin test*: Half a gram (0.5g) of extracts were collected placed in the test tube and 5ml of distilled water were added and was shaped vigorously or place in the steam bath for 5 minutes presence of saponin were seen.
- **Resin test:** Half a gram (0.5g) of extract was measured 5 ml of ethanol plus 4ml of 1% HCl was added and then heated at temperature at about 65 °C for 1-3 mins. There were presence of precipitates indicating resin.
- **Tannin test:** Ten milliliter (10 ml) of distilled water then it was filtered and divided into two portion then few drops of dangerous reagent flow by Wagner reagent were added.
- **Alkaloid's test:** Exactly 0.5g of extracts was measured, 5ml of distilled water were added then two drops of Hydrogen tetraoxosulphate IV acid and two drops of ferric chloride were also added. Presence of green coloration was seen.
- **Flavonoid test:** Half a gram (0.5g) of extracts were measured, 2 ml of 2% dilute NaOH + conc.$H_2SO_4$ +2 drops of $HH_4$ solution was added follow by 2ml Benedict's reagent and boil for 1min. There was presence of reddish coloration

- **Glycoside test:** Half a gram (0.5g) of extracts were measured, plus 10ml of distilled water were added follow by 5% ferric chloride, green color present
- **Steroid test:** And 0.5g of extracts was measured, plus 5% $NH_4$ were added follow by addition of chloroform. Presence of cloudy, clear and ring formation

### Experimental animals

Male and female mongrel puppies between 1.8 - 2.2 kg were obtained from local breeders in K-Vom Jos South Local Government Area Plateau State. The dogs were housed in the dogs quarantine room under Federal College of Animal Health and Production Technology, Vom for two weeks before the commencement of the experiment. The animals were maintained at standard laboratory condition by 12/12 light and dark cycle, 20 +2 °C temperature and 65+5% humidity. Access to dog's plate for feeding and water. Six puppies both sex were used for the studies consisting of 4 groups of two puppies each.

- Group I**:** The control was treated with water only.
- Group II**:** Male and female puppies were administered 4 mg of *Cola nitida* extract based on the body weight per milligram for period of two weeks.
- Group III: Male and Female were Administered 8 mg of *Cola nitida* extracts based on the body weight per milligram for period of two weeks

The administrations were done orally for a period of two weeks by means of oral cannula.

### Sample preparation

Preparation of sample carried out as described by Salahdeen., *et al*. [29] the seed were dried under shade for 2 weeks and there after reduced to powdered form 500 g of the powder seeds were obtained and modified to 427.5 g of kola nut powered which was dissolved in 14litres of hot (100 °C) distilled water at room temperature (25 °C) for 24 hours. The extracts were collected using cotton wool and evaporated to remove solvent by water bath at 100 °C (steam).

### Biochemical test

The biochemical test was carried out in clinical biochemistry laboratory National Veterinary Research Institute, Vom. To determine the following: Aspartate transferase, Alanine transferase, Total protein, Urea and Creatinine.

### Clinical parameters

Clinical parameters were carried out in veterinary clinic Federal College of Animal Health and production technology, Vom. To determine the following body parameters: Body weight, Body temperature, Pulse rate, Heart beat and Respiratory rate.

### Euthanasia

The animals were euthanized through the introduction of air embolism directly to the heart.

### Data analysis

The method of data analysis use is one-way ANOVA.

## Results

The chemical analysis carried out shows the presence of alkaloids, saponins, resin, flavonoid, glycoside, steroid and tannins.

| S/No, | Test | Results |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Saponins | + |
| 3 | Resin | + |
| 4 | Flavonoid | + |
| 5 | Glycoside | + |
| 6 | Steroid | + |
| 7 | Tannins | + |

**Table 1:** Phytochemical analysis of *Kola nut nitida.*

**Key:** WBC-White Blood Cells; LYMPH- Lymphocyte; MONO-Monocyte; NEU- Neutrophils; RBC- Red blood cells; HGB- Hemoglobin; HCT- Hematocrit

### Body parameters

Significant difference was observed ($p < 0.05$) in male body weight, pulse rate, body temperature, heart beat and respiratory rate of puppy administered 400 mg/kg and 800 mg/kg of aqueous extract of kola nut nitida.

**Key:** ID = Identity; ID No. = Identity Number; AST = Aspartate Transferase; ALT =Alanine Transferase; TP = Total Protein; U = Urea; C = Creatinine

### Hematology result

In white blood cells, lymphocyte, monocyte, neutrophil, red blood cell, hemoglobin and hematocrit shows there is normality in all parameters. For the female and male control, white blood cells, lymphocyte, monocyte, neutrophil, Red blood cell, hemoglobin and hematocrit shows there is normality in all parameters (Normocytic). For the female and male administered with 400mg/kg and 800mg/kg of the extract shows hypochromic condition. In white

| Parameter | Body weight (Kg) | Pulse rate | Body Temp. (°C) | Heartbeat (bpm) | Respiratory rate |
|---|---|---|---|---|---|
| Female control puppy | 2.20 ± 0.001* | 78.85 ± 1.935* | 37.92 ± 0.112* | 84.50 ± 1.492* | 24.86 ± 0.455* |
| Female extract (400 mg) | 1.90 ± 0.027* | 65.57 ± 1.312* | 37.21 ± 0.226* | 68.07 ± 22.06* | 9.78 ± 0.853* |
| Female extract (800 mg) | 1.98 ± 0.039* | 63.92 ±1.672* | 36.89 ± 0.281* | 67.43 ± 2.792* | 15.50 ± 0.747* |

**Table 2:** Result showing body weight, pulse rate, body temperature, heartbeat and respiratory rate of female administered 400 mg/kg and 800 mg/kg of kola nut nitida.

*Values with asterisks are statistically significant ($p < 0.05$) compared to control; Temp. = Temperature

| Parameter | Body weight (Kg) | Pulse rate | Body Temp. (°C) | Heartbeat (bpm) | Respiratory rate |
|---|---|---|---|---|---|
| Male control puppy | 2.40 ± 0.001* | 74.42 ± 2.333* | 37.62 ± 0.156* | 80.79 ± 2.139* | 22.93 ± 0.867* |
| Male extract (400 mg) | 1.70 ± 0.027* | 67.07 ± 1.302* | 37.19 ± 0.206* | 68.07 ± 2.137* | 17.71 ± 1.066* |
| Male extract (800 mg) | 1.78 ± 0.039* | 64.64 ± 1.578* | 36.89 ± 0.222* | 67.43 ± 2.968* | 15.79 ± 0.757* |

**Table 3:** Result showing body weight, pulse rate, body temperature, heart beat and respiratory rate of male administered 400 mg/kg and 800 mg/kg of kola nut nitida.

* Values with asterisks are statistically significant ($p < 0.05$) compared to control.

| Parameters | Before treatment | After treatment |
|---|---|---|
| White Blood Cells | 17016.67 ± 1175.77 | 9408.33 ± 2 341.67* |
| Monocyte | 59.00 ± 3.715 | 62.00 ± 1.915$^{NS}$ |
| Red Blood Cells | 4.57 ± 0.112 | 1299.56 ± 823.80* |
| Pack Cell Volume | 32.17 ± 0.477 | 28.17 ± 1.869$^{NS}$ |
| Neutrophils | 39.67 ± 3.333 | 38.00 ± 4.739 $^{NS}$ |
| Lymphocyte | 2.00 ± 0.365 | 3.33 ± 0.667 $^{NS}$ |
| Haemoglobin | 12.83 ± 1.345 | 8.65 ± 0.922 $^{NS}$ |

**Table 4:** Hematological parameters for animals administered 800 mg/kg body of kola nut nitida. Taken before and after treatment.

* Values with asterisks are significantly different ($p < 0.05$) compared to control

$^{NS}$ mean no significant difference.

blood cells and Red blood cell significantly difference was observed ($p < 0.05$) while monocyte, packed cell volume, lymphocyte and haemoglobin there was no significant difference.

## Clinical Signs

Excitement, polyuria, shivering of the stomach, dull appearance, rough hair coat, emaciation, drowsiness, sunken of eye lid, titanic convulsion was seen in the dogs that were administered with 400 mg of the kola nut extract, while severe emaciation, drowsiness, weakness, rough hair coat, aggressiveness, irritating mood, bloody diarrhea were seen in the dogs that were administered with 800 mg of the kola nut extract.

| S/N | ID | ID No. | AST U/L | ALT U/L | TP g/L | Urea mg/dL | Creatinine µmmol/L |
|---|---|---|---|---|---|---|---|
| 1 | Female control puppy | F1 | 38.65 | 29.00 | 37.20 | 32.44 | 0.56 |
| 2 | Male control puppy | E2 | 36.85 | 27.00 | 39.89 | 23.05 | 0.45 |
| 3 | Female extract (400 mg) | C1 | 61.68 | 14.00 | 44.73 | 59.50 | 0.78 |
| 4 | Male extract (400 mg) | C2 | 63.54 | 16.00 | 46.80 | 62.45 | 0.80 |
| 5 | Female extract (800 mg) | D1 | 74.94 | 18.00 | 73.57 | 65.86 | 0.62 |
| 6 | Male extract (800 mg) | D2 | 76.85 | 20.00 | 75.89 | 67.23 | 0.70 |

**Table 5:** Showing biochemical effects of aqueous extract of kola nut *nitida* administered 400 mg/kg and 800 mg/kg.

**Figure 1:** (A) Weakness and sunken of eyeball (B) Mucoid watery faece.

## Gross Lesions

At postmortem, Atrophied testicles and kidney 4/6, Pale lungs 4/6, Enlarged liver, lymph nodes and spleen 4/6, Distended/ engorged gall bladder 4/6, Necrotic foci on the spleen, kidney and liver 2/6, slight congestion of the meninges 2/6 were seen as shown.

**Figure 2:** Engorged gall bladder.

**Figure 3:** Enlarged liver with necrotic foci (Left) and normal liver (Right side).

**Figure 4:** Enlarged lymph node (Left) normal lymph node (Right).

**Figure 5:** (A) Pale lungs (B) Normal lungs.

**Figure 6:** (A) Pale kidney (B) Normal kidney.

**Figure 7:** (A) Slight congestion of the meninges (B) Normal brain.

## Histopathology

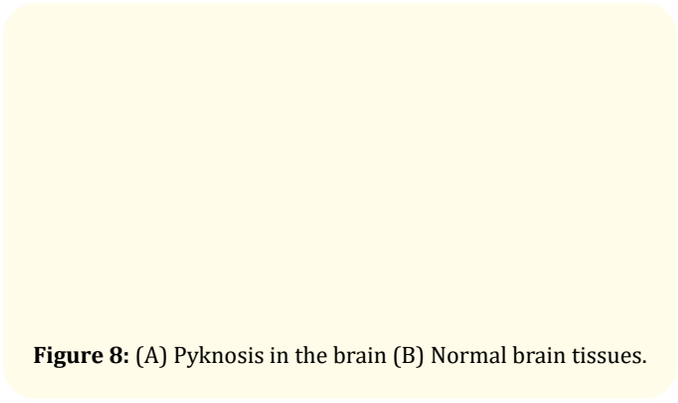

**Figure 8:** (A) Pyknosis in the brain (B) Normal brain tissues.

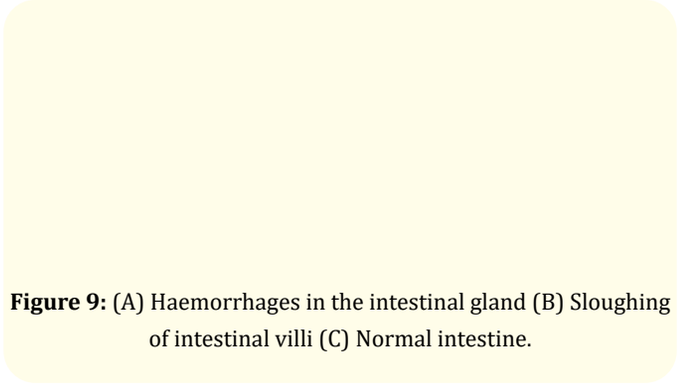

**Figure 9:** (A) Haemorrhages in the intestinal gland (B) Sloughing of intestinal villi (C) Normal intestine.

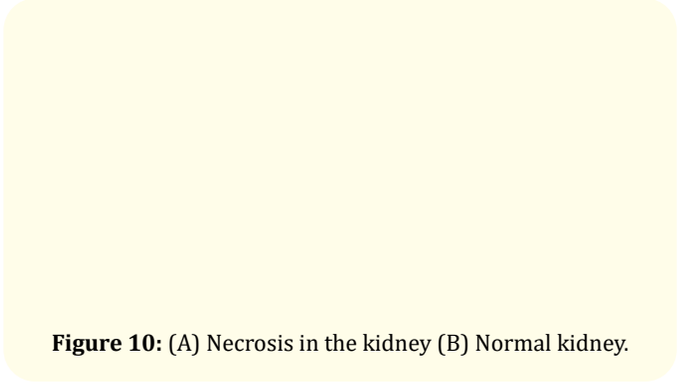

**Figure 10:** (A) Necrosis in the kidney (B) Normal kidney.

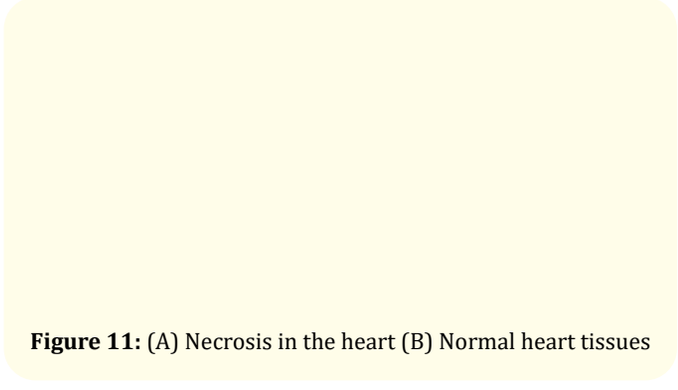

**Figure 11:** (A) Necrosis in the heart (B) Normal heart tissues

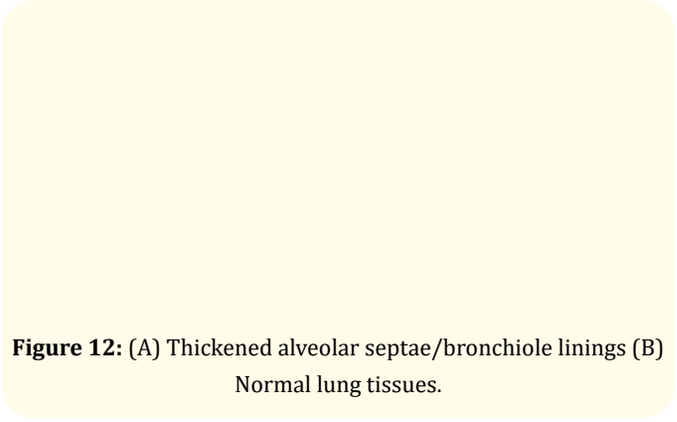

**Figure 12:** (A) Thickened alveolar septae/bronchiole linings (B) Normal lung tissues.

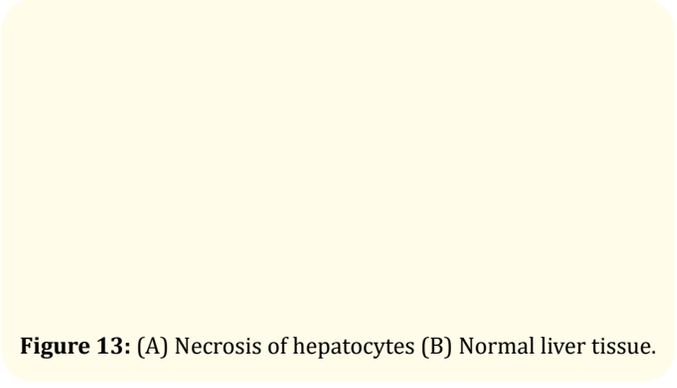

**Figure 13:** (A) Necrosis of hepatocytes (B) Normal liver tissue.

## Discussion

The kola nut nitida chemical analysis carried out shows the presence of Alkaloid, Saponin, Resin, Flavonoid, Glycoside, Steroid and Tannins (Table 1). This is similar to the report of Burdock., *et al*. [1]. The clinical signs finding from this study indicate that different doses of aqueous extract of kola nut nitida (400 mg/kg and 800 mg/kg) can be toxic to dogs as the following clinical signs were observed: body weight lost, decreased pulse rate, decreased body temperature, decreased respiratory rate, sunken eyeball and mucoid diarrhea (Table 2, Table 3 and Figure 1). This study is in agreement with Elisavet., *et al*. [30] which says that the mean lethal dose for caffeine and theobromine is within 100-500 mg with mild clinical signs such as vomiting, hyperactivity, diarrhea, polyuria and restlessness, emaciation, (Figure 1). In the cause of administration of aqueous extract of kola nut nitida on puppies, it shows that in normal saline administration to dogs has no effect on them. But in different dosage given to the puppies, it shows significant effect on them, in 800 mg dose given to both male and female puppies shows the following clinical signs as at third day of administration which is mucoid diarrhea, loss of appetite shivering, excitement, slightly dull appearance, while in 400 mg dosage, excitement, shivering and dullness in appearance was observed after 1hour of administration. The temperature of the entire experimental animal both the control was at the normal range as at the third day.

Fourth days of administration of kola nut extract the puppies under 800 mg, there a reduction body weight of the male from 2.2 kg to 2.0 kg and female 2.0 kg to 1.8 kg, meanwhile the actual weight of male control is 1.4 kg. There was a hypothermia in male and female extract of 800 mg which is 35.5 °C and 35.8 °C (normal range 37.5 - 39 °C). While that of 400 mg was 36.5 °C to 36.8 °C from normal. Pulse rate of 800 mg and 400 mg kola nut extract deviate from 60 - 62 mean while normal range is 65 - 90. Respiratory rate of both 400 mg experimental dogs were at the normal range which is from 15 -30 was observed at 1-2 hours after the administration. While that of 800 mg deviated from 15 - 30 to 14 in both puppies.

At seventh to Tenth days of administration of kola nut extract, there was changes in 800 mg and 400 mg both male and female extract which is drowsiness, restlessness (alertness), mucus diarrhea, irritating mood, sunken of eye pupil, severe emaciation, rough hair coat, shivering and slight bloody diarrhea was observed expect bloody diarrhea in 400 mg. The temperature of 800 mg and 400 mg extract fell below range (hypothermic) which is 36.0°C-36.1°C of both experimental animals, while there heartbeat also fell below normal which shows from 58 bpm-64 bpm normal range is 70-120 bpm. Pulse rate in both experimental animals there was a variation which was observed. Respiratory rate in 800mg and 400 mg there was also variation in value.

From eleventh to fourteenth, in 800 mg extract there was severe emaciation, rough hair coat, reduction in body weight, hypothermic, drowsiness, restlessness (alertness), aggressive mood, sunken of eye pupil, dullness, weakness, jerking and bloody stool. While in 400 mg sunken of eye pupil, dullness, weakness, drowsiness, restlessness (alertness) aggressive mood and rough hair coat. This finding is in agreement with the report of Alvares., *et al*. [31]. After the administration of kola nut extract there was no death recorded but the animals was euthanized by the introduction of air embolus/cardiac punched.

Hematology result showed that there is a statistical significant difference in Red Blood cells, white blood cell, Monocyte, Pack cell volume, Neutrophils, Lymphocyte and Haemoglobin which shows hypochromic condition (Table 4), while in histopathology neuronal necrosis and pyknosis was seen in the brain, sloughing with hemorrhage in intestine, necrosis of the renal tubules in the kidney, necrosis in the myocardium of the heart, thickening of the alveolar septae in the lungs and in liver necrosis of the hepatocytes. In this case the investigation carried out using aqueous extract of kola nut nitida, is in agreement with Elisavet., *et al*. [30], which says that the food association toxicoses in dog and cats. Further reported that toxic dose of caffeine (OTC pure caffeine) for dog is 100-500 mg/kg. Meanwhile, the result gotten from biochemical test shows that aqueous extract of kola nut nitida has great difference on treatment groups. In both female and male control female shows AST 38.65 U/L, ALT 29.00 U/L TP 37.20 g/L Urea 32.44 mg/dl Creatinine 0.56 ummol/L while male control shows AST 36.85 U/L ALT 27.00 U/L TP 39.89 g/L UREA 23.05 mg/dl and Creatinine 0.45 ummol/l compared to both male and female treatment group (400 mg/kg and 800 mg/kg). In female extract (400 mg), AST 61.68 U/L, ALT 14.00 U/L TP 44.73 g/L Urea 59.50 mg/dl Creatinine 0.78 ummol/L while male extract (400 mg) shows AST 63.54U/L ALT 16.00 U/L TP 46.80 g/L Urea 62.45 mg/dl and Creatinine 0.80 ummol/l Female extract 800 mg show AST 74.94 U/L, ALT 18.00 U/L TP 73.57 g/L Urea 65.86 mg/dl Creatinine 0.62 ummol/L while male 800 mg shows AST 76.85 U/L ALT 20.00 U/L TP 75.89 g/L Urea 67.23 mg/dl and Creatinine 0.70 ummol/l.

The toxic effect of aqueous extract of kola nut, show a great threats to some domestic animals such as dogs and cats because their metabolic rate is more slowly compared to that of human. In Fig.4.6a, which shows the presence of neuronal necrosis and pyknotic nuclei on the brain. Fig.4.6b, Male and Female (800 mg) extract shows more of the toxicity of the kola nut. Were by the epithelial lining show severe erosion indicating hemorrhagic and sloughing of the villi. While in Fig.4.6d, the kidney shows mild and slight coagulative necrosis of the tubular epithelial cells. Fig.4.6e, the cardiac muscle found been necrotized compared to that of control. In Fig.4.6f, the lungs show great pathological changes whereby the alveolar septa were thickening more compare to that of control. While in Fig.4g, Male and Female extract shows the presence of necrosis on the hepatocytes.

## Conclusion

This study showed that aqueous extract of *Cola nitida* has adverse effect on puppies such as clinical signs, body parameters, gross lesions, biochemical parameters and finally histopathological lesions.

## Recommendations

Some food that can possess risk of toxicity on domestic pet such as caffeine, theobromine and theophylline containing in kola/chocolate products should not be fed to them. Affected pet should be immediately seek veterinary attention. Decontamination measure especially in the case of emesis, when need arises as well as specific treatment should be given. Proper education of companion animal owners for the risk of food poisoning so as to contribute to the prevention of such incident.

## Bibliography

1. Burdock GA., *et al*. "Safety assessment of kola nut extract as a food ingredient". *Food and Chemical Toxicology* 47 (2009): 1725-1732.

2. Veronique G. "The little-known nut that gave Coca-Cola its name". BBC News (2016).

3. Heckman MA., *et al*. "Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters". *Journal of Food Science* 75 (2010): 77-87.

4. Darragh A., *et al*. "Adverse effects of caffeine". *Iranian Journal of Medical Sciences* 150 (1981): 47-53.

5. Peters J. "Factors affecting caffeine toxicity: a review of the literature". *The Journal of Clinical Pharmacology* 7 (1967): 131-141.

6. Berger AJ and Alford K. "Cardiac arrest in a young man following excess consumption of caffeinated "energy drinks"". *Medical Journal of Australia* 190 (2009): 491-493.

7. Forman J., *et al*. "Myocardial infarction resulting from caffeine overdose in an anorectic woman". *Annals of Emergency Medicine* 29 (1997): 178-180.

8. Campana C., *et al*. "Caffeine overdose resulting in severe rhabdomyolysis and acute renal failure". *American Journal of Emergency Medicine* 32 (2014): e3-4.

9. Mrvos RM., *et al*. "Massive caffeine ingestion resulting in death". *Veterinary and Human Toxicology* 31 (2014): 571-572.

10. Wrenn KD and Oschner I. "Rhabdomyolysis induced by a caffeine overdose". *Annals of Emergency Medicine* 18 (1989): 95-97.

11. Yamamoto T., *et al*. "Autopsy report for a caffeine intoxication case and review of the current literature". *Journal of Toxicologic Pathology* 28 (2015): 33-36.

12. Foor J and Stowe CM. "Acute fatal caffeine toxicosis in a dog". *Journal of the American Veterinary Medical Association* 167 (1975): 379.

13. Michael SJ. "Caffeine poisoning in a dog". *Journal of the American Veterinary Medical Association* 139 (1961): 559.

14. Tawde SN., *et al*. "Death by caffeine: presumptive malicious poisoning of a dog by Incorporation in ground Meat". *Journal of Medical Toxicology* 8 (2012): 436-440.

15. Hensel M., *et al*. "Fatal caffeine intoxication in a dog". *Brazilian Journal of Veterinary Pathology* 10 (2017): 65-68.

16. Handl S and Iben C. "Foodstuff toxic to small animals-a review". *Veterinary Medicine* 95 (2008): 235-242.

17. Kovalkovičová N., *et al*. "Some food toxic for pets". *Interdisciplinary Toxicology* 2 (2009): 169-176.

18. Gugler K., *et al*. "Hidden dangers in the kitchen: common foods toxic to dogs and cats". *Toxicology Compendium* 35 (2013): E1-7.

19. Caloni F and Cortinovis C. "Household food items toxic to dogs and cats". *Frontiers in Veterinary Science* 2 (2016): 26.

20. Jasani S. "Toxicological Emergencies". In: Nind F, editor. *Small Animal Emergency Medicine* (2011): 22-7.

21. Gwaltney-Brant S. "Chocolate intoxication". *Veterinary Medicine* 98 (2001): 108-111.

22. Agudelo CF., *et al*. "Chocolate ingestion induced non-cardiogenic pulmonary Oedema in a puppy: a case report". *Veterinary Medicine* 2 (2003): 109-112.

23. Sudhakara RB., *et al*. "Chocolate Poisoning in a Dog". *International Journal of Veterinary Health Science and Research* 1 (2013): 16-17.

24. Baile ME and Garland T. "Toxicologic emergencies". In: Reinhardt RW, editor. *Veterinary Emergency and Critical Care Medicine*. Mobsy, St. Louis (1992): 427-452.

25. Poppenga RH. "Toxicological emergencies". In: Al. KLS et, editor. *BSAVA Manual of Canine and Feline Emergency and Critical Care* (2007): 278-294.

26. Meola SD. "Chocolate toxicity". In: Mazzafero EM, editor. Blackwell's Five-Minute Veterinary Consult Clinical companion. Small animal emergency and critical care. *Blackwell Publishing. Ames* (2010): 141-145.

27. Mancitire DK., *et al*. "Toxicologic Emergencies". In: Troy DB, editor. Manual of Small Animal Emergency and Critical Care Medicine. *Lippincott Williams and Wilkins. Baltimore USA* (2005): 384-387.

28. Adisa WA., *et al*. "Effects of aqueous extract of Kola Nut (Cola nitida Rubra) on reproductive hormones in Rats". *Nigerian Journal of Physiological Sciences* 25 (2010): 121-123.

29. Salahdeen HM., *et al*. "Gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extracts of *kolanut* (*Cola nitida*) (vent) and its toxicity studies in rats". *Journal of Medicinal Plants Research* 9 (2015): 56-70.

30. Elisavet WK., *et al*. "Dog domestication and the dual dispersal of people and dogs into the Americas". *In: Proceedings of the National Academy of Sciences* 118.6 (2021): e2010083118.

31. Alvares F., *et al*. "Old World Canis spp. With taxonomic ambiguity: Workshop conclusions and recommendations". In: CIBIO. Vairão, Portugal (2019).