

# Caffeine modulates biliary secretions in indigenous Nigerian dogs

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**Abstract:** This study was aimed at evaluating the effect of caffeine on biliary secretions in indigenous Nigerian dogs. A total of 6 dogs weighing 12 – 15 kg divided into 2 groups were used. The control group was fed their normal diet and water *ad libitum* and the treated group received 16 mg/kg of white crystalline caffeine dissolved in 10 mls of water and administered orally 8 hours prior to each surgery. Under sodium thiopentone and ketamine anaesthesia, common bile duct cannulation was done by the modified method of Rath and Hutchison. Bile was collected immediately post cannulation over a period of 48 to 72 hours. The bile samples from all dogs were analysed for bile volume, pH and electrolyte concentrations. The results showed significant increase in the bile volume in the caffeine treated group:  $3.41 \pm 0.85$  ml compared to the control group:  $1.24 \pm 0.17$  ml ( $p < 0.05$ ). The bile pH in the caffeine treated group:  $7.40 \pm 0.24$  was significantly higher than the control group:  $6.68 \pm 0.18$  ( $p < 0.05$ ). The potassium concentration of  $6.08 \pm 0.49$  mmol/L in control group was significantly higher than the potassium concentration of  $4.81 \pm 0.21$  mmol/L in the treated group ( $p < 0.05$ ). However, there was no significant change in the concentration of bicarbonate, chloride and sodium ions in the caffeine treated animals. We conclude that orally administered caffeine significantly increased bile volume and bile PH and significantly decreased bile potassium concentration in indigenous Nigerian dogs and these findings may have implication for digestion and absorption of fat soluble vitamins and a measure of liver functions.

**Keywords:** Caffeine, Bile Volume, Bile Secretion, Liver Function, Fat Digestion and Absorption, Indigenous Nigerian Dogs

## 1. Introduction

The liver is the largest gland in the body and one of its principal functions is the formation of bile which is required for the digestion and absorption of fat and fat soluble vitamins, elimination of detoxified drugs and metabolites (Lautt, 1997).

Bile is secreted by the hepatocytes into the biliary canaliculi and modified in the bile ductules and the gallbladder. It is an alkaline aqueous solution of bile acids, cholesterol, phospholipids, bile pigments, lecithin and inorganic electrolytes (Burwen *et al.* 1992, Barabote *et al.* 2006).

Several factors are reported to affect the rate and compositions of biliary secretions, for example neural – vagal and sympathetic stimulation (Farouk *et al.* 1992), local

hormonal factors such as secretin (Nyberg, 1990), serotonin and serotonin antagonists, bile salts, protein and fatty meals, duodenal luminal factors such as hyperosmolar saline, calcium and light mucosal stroking (Leonard *et al.* 1981).

Other factors modulating biliary secretion include tri-iodotyronine - T3 (Gebhard and Prigge, 1992), cysteamine (Omar *et al.* 2005) neuropeptide Y (Farouk *et al.* 1992), somatostatin (Bergstrom and Thulin, 1989, Nyberg, 1990), vasoactive intestinal polypeptide – VIP (Nyberg *et al.* 1990) and circadian rhythms (Nakano *et al.* 1990, Duane *et al.* 1979).

Caffeine is a bitter crystalline xanthine alkaloid and the world's most widely consumed psychoactive substance that is unregulated in nearly all jurisdictions (Lovett, 2005). Caffeine is present naturally or as additive in widely consumed foods and beverages such as kolanut (particularly

in northern Nigeria and savannah Africa), coffee, tea, chocolates/candies and assorted soft drinks (Juiano and Griffiths, 2005., The Vaults of Erowid, 2006. ).

WHO in 2003 reported that 21.7% of the world population consume coca cola which contains 34mg of caffeine per serving (355ml) or 96mg per litre. Unhealthy diet has been implicated in the development of liver diseases and evidence has shown that excessive consumption of "soft drinks" pose an increased risk for cardiometabolic risk factors, metabolic syndrome and liver cirrhosis similar to that seen in chronic alcoholics (Dhingra et al. 2007, Zelber-Sagi *et al.*, 2007).

Consequently, this study was designed to study the possible effect of orally administered caffeine on bile secretion in indigenous Nigerian dogs.

## 2. Materials and Method

### 2.1. Experimental Animals

Nine indigenous Nigerian dogs purchased from the dog market in Giwa Local Government Area of Kaduna state of Nigeria were humanely transported in a vehicle to the Department of Veterinary Surgery and Medicine and Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria. They were housed (conditioned, acclimatized and maintained) in the dog kennels throughout the period of study. They were fed rice, maize, soft bones and clean water provided *ad libitum*.

### 2.2. Ethical Consideration

The animals were humanely handled according to Ahmadu Bello University guidelines on care and handling of laboratory animals and in accordance to Helzinki's declaration.

### 2.3. Pre-Operative Evaluation

Physical examinations were performed on all dogs, accompanied by laboratory screening of faeces and blood at Clinical Pathology, Protozoology and Haematology Laboratories at Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria. Successfully screened animals were grouped and entered for the surgical procedures. Each dog's abdomen was shaved and pre-scrubbed using Chlorhexy povidone swabs before anaesthetic induction and endotracheal intubation.

### 2.4. Study Design

Six healthy adult indigenous Nigerian dogs of non-specific sex and age weighing 12 – 15 kg were used for the study. The 6 dogs were arranged into two experimental groups of 3 dogs each and accommodated in a separate kennel. The groupings were as follows:

Group A (Control): Dogs were fed their normal diet with 10mls of normal saline orally, water and food *ad libitum*.

Group B (Caffeine treated); received 16 mg/kg of white crystalline caffeine (BDH, England, Batch no 6374170)

dissolved in 10mls of normal saline orally 8 hours prior to surgery. Food and water was allowed *ad libitum*

### 2.5. Anaesthetic Procedure

Each dog was premedicated with atropine 0.05 mg/kg subcutaneously and chlorpromazine 4 mg/kg s/c (Van-Wijk *et al.*2001). Sodium Thiopentone was administered at a rate of 15 mg/kg dose to effect by continuous intravenous infusion. Anaesthesia was maintained with ketamine hydrochloride 10 mg/kg i.m. Vital signs were monitored and charted. Following anaesthetic induction, the dogs were intubated and restrained in dorsal recumbency.

### 2.6. Surgical Procedure

Surgery site was draped in a rectangular draping pattern. A 16 cm cranial mid-ventral abdominal incision was made from approximately 2 cm to the xiphoid process extending to approximately 8 cm cranial to the umbilicus to access the peritoneal cavity. The omentum was used to pack the intestinal segment and padded with saline soaked gauze. The duodenal loop was identified, mobilized caudally while the liver lobes were lifted to expose the bile ducts which were traced to the gall bladder. All exposed viscera surfaces were kept moist with warm saline soaked gauze. Two preplaced vincyrl 2/0 stay-sutures were used to raise the bile duct to adequately expose the surgical site. A cannulation site was selected avoiding the great hepatic (portal) vessels and duodenal mesenteric vessels and approximately at a distal one-third to catheterize the bile duct. A stab incision was made on the bile duct to allow introduction of a 5 Fr feeding tube, and fixed with two interrupted stitches using vicryl 3/0. This was to avoid slippage. The catheter was passed trans abdominally and fixed to the right lateral abdominal peritoneal wall lining. This precaution was undertaken to prevent dislodgement of the catheter and spillage of bile intraperitoneally. Upon catheterization, bile was seen as yellowish fluid flowing through catheter lumen. The catheter was corked using the stopper which was released during bile collection. (In-flow of bile in the catheter confirmed adequate catheterization) Intraperitoneal cleaning of minimally spilt bile with warm saline soaked gauze was done thoroughly. The above procedure was carried out according to the modified method of Rath and Hutchison, (1989). Bile flow and sample collection continued for 48 to 72 hours in all cases that underwent the operation. Postoperative collection of bile was by clean sterile test tubes and the samples were stored at refrigerating temperature of 4 °C.

Abdominal viscera were rearranged for abdominal closure in 3 layers (peritoneum and subcutaneous using vicryl 2/0 in simple continuous suture pattern while skin closure was done using nylon 2/0 in horizontal suture pattern). Post-operative wound healing was assisted using topical povidone iodine ointment. This was applied daily after adequate cleaning and dressing of the wounds. All abdominally created wounds were dressed occlusively using gauze and adhesive tapes.

**2.7. Bile Collection and Analysis**

Bile samples collected over a period of 72 hours were pooled in sterile sample bottles for each dog. Samples were stored immediately at 4°C in a refrigerator until the time of the assay. Usually, the samples were analysed within 48 to 72 hours post collection for bile pH and electrolyte concentrations. The bile volume was measured in ml/24 hours taken 8 hourly and pooled. Biochemical analysis was done at Chemical Pathology Department, Ahmadu Bello University Teaching Hospital, Shika, Zaria, Nigeria. The bile samples were analysed by the Flame photometric method, Titrimetric method and Audicom electrolyte analyser, model: Ac 9900, (made in Germany) for sodium, potassium, chloride, bicarbonate and pH concentrations in bile using the principle of ion selective electrodes.

**3. Statistical Analysis**

The data obtained from the experiment was expressed as Mean ± SEM, and analyzed by unpaired student t- test using SPSS statistical software (version 17.0) for comparison between two groups. P < 0.05 was considered as statistically significant.

**4. Results**

**4.1. Effect of Caffeine on Bile Volume**

The mean bile volume of 1.24 ± 0.17ml in the control

group was significantly lower than 3.41 ± 0.85ml in the treated group (p< 0.05). Administration of caffeine orally lead to a sharp rise in volume of bile secreted which became noticeable before 16 hours while there was a gradual decline in volume of bile secreted in the control group. See table 1 and figure 1s.

**4.2. Effect of Caffeine on Bile pH Concentration**

Results obtained for mean pH concentration were 6.68 ± 0.18 and 7.40 ± 0.24 for the control and treated groups respectively. The pH concentration in control group was significantly lower than the pH concentration in the caffeine treated group (p< 0.05). The bile pH of the control group decreased sharply (p<0.001) from the 16<sup>th</sup> hours while bile PH in the caffeine treated group remained stable at a higher level over a period of 24 hours. See table 1 and figure 2.

**4.3. Effect of Caffeine on Bile Electrolytes**

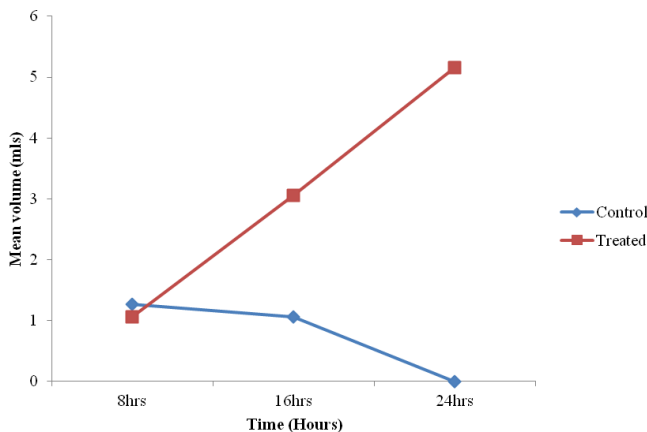
Mean potassium concentration of 6.08± 0.49 mMol/L in control group was significantly higher than the potassium concentration of 4.81 ± 0.21nMol/L in the treated group (p< 0.05). Following oral administration of caffeine, there was no significant change in potassium concentration in the bile until the 16 hours when a statistically significant drop (p<0.001) in the bile potassium concentration of the control animals was noted. See table 1 and figure 3.

There was no significant difference the bile concentrations of sodium, chloride and bicarbonate between the control and caffeine treated dogs.

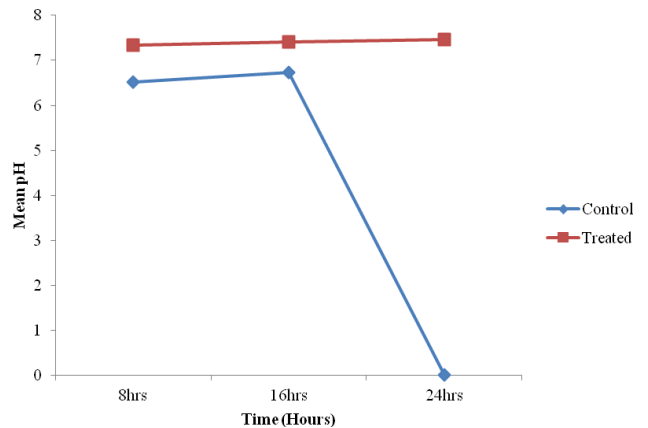
*Table1. Effect of caffeine on bile volume, pH and electrolyte concentrations in control and treated dogs.*

Parameters	Normal range	Control animals.	Caffeine treated animals
Bicarbonate(mmol/L)	18.00 - 25.00	23.00 ± 3.16	23.78 ± 0.91
Chloride (mmol/L)	109.00 - 122.00	96.80 ± 4.62	105.60 ± 7.07
Sodium (mmol/L)	146.00 - 156.00	142.20 ± 17.47	137.40 ± 0.82
Potassium (mmol/l)	3.80 - 5.60	6.08 ± 0.49	4.81 ± 0.21*
Volume (ml/24hrs)	1.0 – 5.0	1.24 ± 0.17	3.41 ± 0.85*
pH	7.00 - 7.80	6.68 ± 0.18	7.40 ± 0.24*

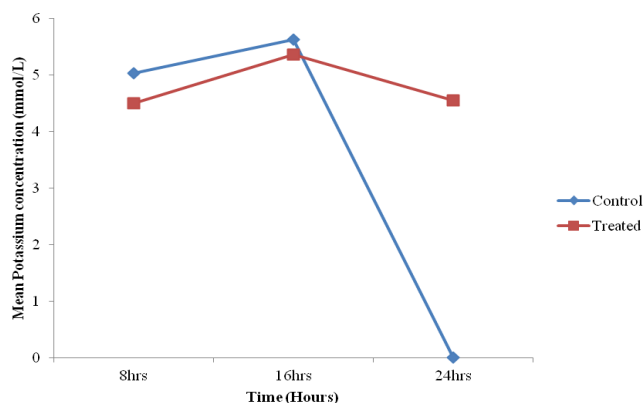
\*= p < 0.05, shows significant difference (p < 0.05). Values are mean +SEM for control and caffeine treated animals



**Figure 1.** Timed effect of caffeine on bile volume of control and treated indigenous Nigerian dogs.



**Figure 2.** Timed effect of caffeine on bile pH of control and treated indigenous Nigerian dogs.



**Figure 3.** Timed effect of caffeine on bile potassium concentration of control and treated indigenous Nigerian dogs.

## 5. Discussion

Bile aids the process of digestion and absorption of lipids and fat soluble vitamins in the small intestine and helps in preventing steatorrhea (Barabote *et al.*, 2006). It also acts as a bactericide destroying microbes like *Helicobacter pylori* and *Salmonella* spp. (McGowan *et al.* 1996, Tennant *et al.* 2008). The human liver produces about one litre of alkaline bile daily depending on body size and amount of food eaten (Bowen, 2001).

From this study, there was a significant increase in the bile volume in caffeine treated group ( $3.41 \pm 0.85$ ml) compared to the control group ( $1.24 \pm 0.17$ ml). This suggests that orally administered caffeine has a choleric action. Omar *et al.* (2005) has reported increase in basal bile secretion within the first 30-60 minutes following bile cannulation in rat. Cysteamine was also reported to cause a significant decrease in bile flow (Omar *et al.* 2005)

The major regulator of the rate of production of hepatic bile is the return of bile salts to the hepatocytes via the enterohepatic circulation which provides the driving force for fluid transport into the biliary system (Pocock and Richards, 1981, Ganong, 2005). Cannulation of the common bile duct, as done in this study, interrupts enterohepatic circulation of bile salts and the volume of bile secreted is equal to the rate of bile synthesis by the hepatocytes (Gebhard and Prigge, 1992).

Bile secretion is also subject to central modulation (Farouk *et al.* 1992). Caffeine is reported to cross the blood brain barrier and acts as a nonselective antagonist of adenosine receptor by competitive inhibition (Fisone *et al.* 2004) and may therefore stimulate bile secretion through vagal stimulation. Another possible central mechanism of choleric action of caffeine may be inhibition of central somatostatin and or alterations in central somatostatinergic neurotransmission. Somatostatin acts as a putative neurotransmitter and or neuromodulator and known to regulate release of several hormones and neuropeptides, inhibiting biliary secretion and altering bile constituents in experimental animals and man (Bergstrom and Thulin, 1989 and Nyberg, 1990).

Locally, Serotonin (5-HT) is known to enhance bile secretion. Administration of p-chlorophenylalanine, a 5-HT synthesis blocker and 5, 7-hydroxytryptamine (5-HT neurotoxin) have been reported to cause a decrease in biliary secretion (Leonard *et al.* 1981). Therefore, orally administered caffeine may increase serotonin release and increase the rate of bile synthesis as observed by increased bile volume in this study.

The normal pH of bile in humans ranges between 7.0 - 7.8 (Bowen, 2001) and is greater than 6 in dogs (Steve and Roger, 2006). The alkaline bile neutralizes the acidic chyme entering the duodenum and activates pancreatic lipases to further improve digestion and absorption of lipids and may prevent steatorrhea and formation of biliary calculi (Bowen, 2001, Steve and Roger 2006 and Sai *et al.* 2010).

From our study orally administered caffeine significantly increased bile PH to  $7.40 \pm 0.24$  from  $6.68 \pm 0.18$  in the control group. The secretion of bicarbonate ( $\text{HCO}_3$ ) rich fluid by the ductal cells into the bile is controlled by secretin, glucagon and gastrin and significantly contribute to the alkalinity of bile (Pocock and Richards, 2006 and Maldonado-Valderrama *et al.*, 2011). Caffeine may act directly on the ductal cells or by increasing secretion of the local intestinal hormones. The production of increased quantity of alkaline bile enhances the activity of pancreatic lipase in the intestine which ultimately improve digestion of fat.

Bile salts are formed within hepatocytes and secreted as sodium and potassium salts of bile acids (Steve and Roger, 2006). In this study, potassium concentration was significantly lowered in the caffeine treated animals ( $4.81 \pm 0.21$ mmol/L). Although the value is within the normal range of 3.80 - 5.60 mmol/L (Duncan and Prasse, 2011), bile-acid-independent bile secretion involves secretion of water and electrolytes by the hepatocytes and the ductal epithelial cells, where sodium is transported actively into the bile canaliculi with passive movement of chloride ion and water (Pocock and Richards, 2006).

The active secretion of bicarbonate into the bile by ductal cells is followed by passive movement of sodium and water. In the gallbladder,  $\text{Na}^+ - \text{K}^+$  ATPase is abundantly distributed in the basolateral surfaces of the epithelial cells and cause reabsorption of sodium, chloride and bicarbonate into the epithelial cells and secretion of potassium into the bile. The initial rise in bile potassium is subsequently reversed because of passive diffusion back into the cells along the concentration gradient already established (Leonards *et al.*, 1981, Pocock and Richards, 2006). Caffeine may enhance passive reabsorption of potassium into the epithelial cells or alter the activity of the  $\text{Na}^+ - \text{K}^+$  pump.

From this study, there was no significant change in the concentration of bicarbonate, chloride and sodium ions in the caffeine treated animals. This will suggest selective inhibitory action of caffeine on secretion of potassium ion by the ductal cells into the bile, by altering the activity of  $\text{Na}^+ - \text{K}^+$  ATPase.

We conclude that orally administered caffeine significantly increased bile volume and bile PH and significantly decreased bile potassium concentration in indigenous Nigerian dogs, possibly by central and local mechanisms, and may improve digestion and absorption of fat and fat soluble vitamins.

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