Effect of Nescafe 3 in 1™ and Cowbell ™ Sachet Coffees on Body Weight, Urea, Creatinine, Lipid and Enzyme Profiles and Electrolytes in Healthy Albino Wistar Rats

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ABSTRACT

Background and Objectives: Coffee the source of caffeine is consumed globally. The amount of caffeine in food product varies depending upon the serving size, type product and production method (Elmaadawy et al., 2015). Caffeine, one of the several ingredients in foods is capable of exerting a physiological and pharmacological effect. Nescafe is a brand of instant coffee made by Nestle. It comes in different product forms (Elmaadawy et al., 2015). Nescafe 3 in 1™ Ghana PLC and Cowbell Promasidor Nigeria Limited Coffee are brands of coffee widely consumed in Nigeria. From a public health point of view little seems to be understood about the health benefits. The purpose of this work is to evaluate the effect of Nescafe 3 in 1™ and cowbell ™ coffees on body weight, serum urea, creatinine, lipid, and enzyme profile in healthy rats.

Material and Methods: Thirty healthy male albino rats (150±5.35g) were assigned into five groups of six rats. Group 1 served as control fed with basal diets; groups 2 and 3 Nescafe 3 in 1 high dose, 770mg/kg, and Low dose, 510mg/kg of body weight. Group 4 and 5 cowbell coffee high doses, 620mg/kg and low dose 310mg/kg. All administration was done orally, water and food given ad libitum. The treatment lasted for 30days. At the end of the period, the animals were sacrificed and blood samples were collected for analysis.

Result: Nescafe 3 in 1™ low dose cause significant ($p<0.01$) increase in body weight while cowbell coffee ™ treated groups caused a decrease in body weight. There was an increase in AST, creatinine, HDL, serum urea, K^+, HCO_3^- levels in Nescafe 3 in 1™ while Na^+, Cl^- were decreased in cowbell coffee ™ compared with control.

Conclusion: Nescafe 3 in 1™ coffee caused increased body weight although the high dose group was not statistically significant while the consumption of cowbell coffee ™ caused a significant decrease. These differences might result from the composition of these coffees especially their coffee content. Further investigation might be necessary on these brand sachet coffees.

1. Introduction:

Coffee beverage is globally consumed and is prepared in a wide variety of formats. Cowbell coffee and Nescafe breakfast 3in1 are leading brands of coffee beverages available to consumers by Promasidor Nigeria Limited and Nestle Ghana Limited. Prepared as a coffee powder from coffee bean plants (Choi & Curhan, 2007), the coffee beverage is the most popular worldwide (Frary et al., 2005). Scientific studies have evaluated the relationship between coffee beverage consumption and a vast array of clinical conditions. Available findings are contradictory as to whether coffee beverage consumption has an adverse effect on health or not.

Several studies found that coffee might increase the risk of chronic diseases. Jee et al., (2001) found that coffee consumption for more than one time daily led to a slight

increase in blood pressure. Similarly, Chown et al., (2001) and Keijzers et al., (2002) found that the consumption of a high amount of coffee resulted in impaired glucose tolerance. It has been reported that high coffee consumption may cause acute myocardial infarction, stroke, (Mostofsky et al., 2010). Some studies revealed that Cafestol and Kahweol esters found naturally in coffee elevate serum activity of alanine aminotransferase (Urgert & Katan, 1997), high cortisol (Wempleet al., 1997), and impaired glucose tolerance. Nevertheless, there are health benefits associated with coffee consumption. It has been observed that coffee beverage consumption may help prevent some chronic diseases including type 1 diabetes mellitus, Parkinson disease, liver cirrhosis and hepatocellular carcinoma. Epidemiological studies indicate that drinking a large amount of coffee drastically reduce the incidence of type 2 diabetes (Carlsson et al., 2004). Caffeine, an active component in coffee has been linked to improving immune function.

Research related to the effect of consumption of cowbell coffee and Nescafe 3 in1coffee sachet on body weight, serum urea, creatinine, lipids, enzyme, and electrolytes are lacking. Therefore, we carried out this study to evaluate the effect of these brands of sachet coffee Nescafe 3 in 1 and cowbell coffee on body weight, serum urea, creatinine, lipids, and enzyme as well as an electrolyte in healthy albino Wistar rats.

2. Materials and Methods:
2.1. Preparation of Animals:
Male Wistar rats (150±5.35g) bred in the Animal House Facility of Faculty of Basic Medical Science, CRUTECH, Okuku Campus, Cross River State, Nigeria, housed under standard conditions, maintained in 12h light/dark cycle and had free access to food and water, at 280C ± 20 and 58% humidity was used. After 7 days of acclimatization, the rats were randomly assigned into five (5) groups of six (6) rats. Group 1 served as control, fed with normal feed. Group 2 and 3 served as the High dose (HD) and Low dose (LD) Nescafe 3 in 1 respectively and group 4 and 5 served as the High dose (HD) and Low dose (LD) Cowbell coffee respectively.

2.2. Preparation and Administration of Coffee Beverages
All coffee brands used in the study were bought from Local Supermarket in Okuku, Yala Local Government Area of Cross River State, Nigeria. Nescafe breakfast 3 in 1, 36g net weight, a product of Nestle Ghana Limited. The low dose(LD) received 510g/kg, while the high dose (HD) received 770mg/kg. Cowbell coffee 22g net weight, a product of Cowbell Nigeria Limited, administered 310mg/kg and 620mg/kg low dose (LD) and high dose (HD) respectively. All administration was prepared fresh and given oral gastrically.

2.3. Weekly Weight Changes
The body weight of each rat was measured using balance model TORSON 80 at the beginning of treatment, the difference between the body weight at the beginning and the weekly treatment represent weekly weight change. These changes were recorded during the experimental period of 30 days.

2.4. Preparation of Serum
The animals were sacrificed and their abdominal regions were opened along the linea alba and the diaphragm was cut with a scalpel blade to expose the heart. Blood was collected by cardiac puncture using 10ml syringes into properly labeled non-heparinized tubes. The blood samples were allowed to stand for 1hr after which they were centrifuged at 3000rpm for 10minutes using Uniscope Laboratory Centrifuge. The serum was aspirated into clean dry sample bottles using Pasteur pipette and was kept in corresponding labeled sample bottle and used within 12hr of preparation as described by (Malomo, 2000) for scientific analysis.

2.5. Analysis
Blood urea nitrogen and creatinine concentration were determined according to the method described by Harrison (1947). Serum analyzed for total cholesterol (TC), triglycerides (TG), HDL were done by enzymatic method coupled with a spectrophotometer using assay kit (Randox Lab Ltd, Co. Antria UK). LDL and VLDL were estimated with the use of Friedewald's formulae (Friedewald et al., 1972). Serum alanine transaminase (ALT) activity was determined by the method of Reitman and Frankel (1957), Aspartate aminotransferase AST was assayed according to the principle described by International Federation of Clinical Chemistry Committee of Standard (1980). Alkaline Phosphate ALP activity by the method of Walter and Schutt (1977).

3. Results:
There was significant (p<0.001) increase in body weight in NSCLD, although the increase in the high dose group was not statistically significant while and the CBC-Treated groups caused decreased in body weight (Figure1) when compared with control following the administration of the beverages. Serum AST, ALP levels were decreased in test group while ALT was increased in test groups compared with control. TC, TG, HDL levels were increased in the high dose NSC while the NSCLD and CBC-treated groups had significant reduction levels (Table 1).

Serum urea levels and Urea/Creatinine ratio increased significantly in both coffees. Nitrogen ratio and Serum creatinine levels were significantly (p<0.001) decreased in both brands of sachet coffees compared with control. Nescafe-fed rats significantly reduced (p<0.01) serum sodium levels while Cowbell-fed rats had increased

serum sodium levels. Serum potassium levels in Nescafe-fed rats were significantly ($p<0.001$) increased while the Cowbell-fed rats had increased serum sodium levels compared with control. The chloride levels and bicarbonate levels showed an inverse relation with decreased levels of chloride and increased levels of bicarbonates compared with control (Table 2).

4. Discussion:

Chronic consumption of coffee beverages (Nescafe breakfast 3in1TM and Cowbell TM) on body weight, serum urea, and creatinine, lipid and enzyme profiles was studied in male rats. The result of body weight showed that NSCLD brands caused increased weight gain while CBC at the doses of 310mg and 620 mg/kg caused decreased weight compared with control. Because the final body weight in the CBC treated groups were significantly lower than the control group, the decreased in body weight might be the result of the beverage intake. Loss of weight might be caused by decreased food intake due to loss of appetite, (Lima et al., 2005).
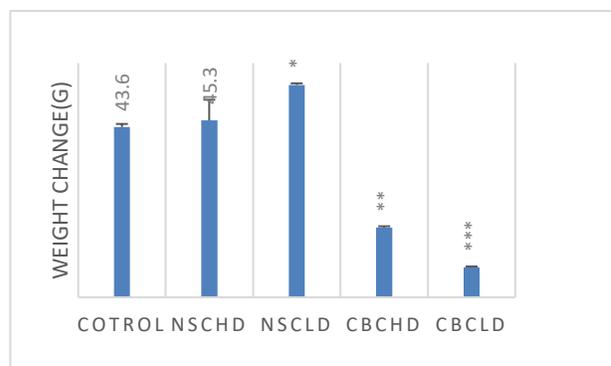

Figure 1. Comparison of body weight changes in different experimental groups of male albino Wistar rats following the administration of Nescafe coffee high dose,(NSC-HD), Nescafe coffee low dose(NSC-LD), Cowbell coffee high dose(CBC-HD) and Cowbell coffee low dose(CBC-LD). Values are express as mean ±SEM. n=6 ***significant at $p<0.001$, **significant at $p<0.01$, *significant at $p<0.05$.

Table 1: *Serum Enzymes, Lipids Profiles In Nescafe 3in1ᵗᵐ And Cowbell ᵀᴹ Coffee Fed-Rats*

	CONTROL	NSC HD	NSC LD	CBC HD	CBC LD
AST (U/L)	153.60± 0.10	133.30±0.02**	147.30±0.02**	149.70±0.02**	147.30±0.03***
ALT (U/L)	135.80± 0.02	136.10±0.04**	137.20±0.04**	142.20±0.02**	139.20±0.02**
ALP (U/L)	147.70± 10.10	109.20±1.45*	137.00±2.72	131.00±7.60*	135.80±2.50*
TC (mg/dl)	1.28±0.02	1.40±0.02*	1.12±0.02**	0.96±0.03***	1.03±0.03**
TG (mg/dl)	1.17±0.02	1.26±0.04*	1.05±0.03*	0.78±0.02**	0.82±0.04***
HDL (mg/dl)	0.32±0.01	0.35±0.01*	0.38±0.01*	0.36±0.01*	0.38±0.02**
LDL (mg/dl)	0.43±0.03	0.35±0.05**	0.30±0.03**	0.33±0.01**	0.35±0.01**
VLDL (mg/dl)	0.52±0.07	0.57±0.02*	0.48±0.01**	0.55±0.01NS	0.37±0.01**

Values expressed as Ẍ± SEM, n=6 *p<0.05. **p<0.01; ***<p0.001 NSC HD=Nescafe coffee high dose, NSCLD=Nescafe coffee low dose, CBCHD=cowbell coffee high dose, C LD=cowbell coffee low dose.

Table 2: *Serum urea, creatinine and electrolytes in Nescafe 3in1ᵀᴹ and cowbell ᵀᴹ coffee fed-rats*

	CONTROL	NSC HD	NSC LD	CBC HD	CBC LD
Serum urea (mg/dl)	2.10±0.004	3.40±0.011***	2.60±0.011**	2.40±0.22**	2.20±0.011*
Serum creatinine (mg/dl)	87.50±0.004	58.70±0.004**	51.50±0.002***	51.20±0.004***	44.40±0.002***
Urea/CR ratio	0.02±0.07	0.05±0.04*	0.05±0.08*	0.04±0.08*	0.04±0.04*
Na^+ (mg/dl)	142.80±0.22	140.80±0.22**	143.40±0.27**	145.20±0.22**	150.20±0.22**
K^+(mg/dl)	5.96±0.02	6.12±0.05***	7.06±0.02**	5.24±0.02***	5.70±0.13NS
Cl^-(mg/dl)	101.60±0.27	100.40±0.20*	103.80±0.22**	106.20±0.22**	106.20±20**
HCO_3^+(mg/dl)	16.40±0.27	21.80±0.22	19.80±0.22**	20.40±0.27**	16.20±0.22NS

Values expressed as Ẍ± SEM, n=6 *p<0.05. **p<0.01; ***p<0.001, NSCHD=Nescafe coffee high dose, NSCLD=Nescafe coffee low dose, CBCHD=cowbell coffee high dose, CBCLD=cowbell coffee low dose.

This is supported by previous findings that caffeine, a biologically active component in coffee reduces energy intake (Tremblay et al., 1988) and has a positive relationship between satiety and daily caffeine intake in men and women (Westerterp-Plantanga et al., 2005) and that subjects that increase their caffeine consumption gain less weight (Lopez-Garcia et al., 2006). Weight as an indicator of health in this study would suggest that significant dehydration occurred in CBC treated rats.

Both brands of coffee caused elevated blood urea in the rat. Several factors may lead to this increase urea such as decreased renal function resulting from compromised renal excretion, liver disease, and dehydration. Increased plasma urea may lead to renal pathology. Serum creatinine levels were significantly lower resulting in a reduced urea/creatinine ratio (DuFour, 1998). Reduced levels of serum creatinine may be caused by the significant reduction of body weight, as serum creatinine ratio is a function of relative muscle mass

The cholesterol raising effect of coffee has been controversial, this study reveals that TC concentration in NSHD group was elevated suggesting concentration effect, whereas in NSLD and CBC treated groups there were significantly reduced serum levels of TC, TG, LDL, and VLDL. Elevated TC levels may be due to diterpenes present in coffee. These diterpenes are said to lower cholesterol through their actions as ligands for farnesoid X and pregnane X receptors on the liver (Ricketts et al., 2007; Alrefai & Gill, 2007; van der Velde et al., 2008). Several findings on the effects of coffee consumption on the components of lipid profiles point out that the use of unfiltered coffee consistently gives negative effects attributed to diterpenes especially Cafestol (Urgert et al., 1997; Grubben et al., 2000). Studies that evaluated the effect of filtered coffee on lipids have had conflicting results, yet other studies have found a positive effect of reducing TC and LDL and increased HDL levels,(Kempf et al, 2010).

Correa et al., (2013) found an increase in both TC as well as the HDL levels after consumption for 4 weeks of filtered coffee.

Chronic consumption of coffee has been associated with increased cholesterol (Jee et al., 2001; increased blood pressure, a strong risk factor for heart disease (Noordzij et al., 2005; Mesas et al., 2011). The beneficial effect of coffee has been studied, the chlorogenic acid content has been reported to give the antioxidant properties of coffee (Svilaas et al., 2004). An inverse association between coffee consumption and markers of inflammation (Lopez-Garcia et al., 2006) as well as the diminished risk of developing diabetes mellitus has been reported (Harris et al., 2007; Huxley et al., 2009).

These brands of coffee contain many other constituents that may affect health. Research has shown that those who drink 2 or more sugar sweetened beverages a day at are a risk of developing gout disease. Aspartame has been associated with eye and ear infection (Frary et al., 2005). Salt has been associated with high blood pressure. Recent studies have linked consumption of coffee with the development of many health hazards such as liver cirrhosis (Klatsky et al., 2006), liver cancer (Bravi et al., 2013), nonalcoholic liver disease (Chen et al., 2014).

Some studies show favorable levels of markers associated with increased coffee intake. In this study, AST levels were significantly decreased and while ALT levels increased in test groups in both brands of coffee. Many researchers proposed that liver enzymes are targets of caffeine found in coffee (Casiglia et al., 1993). Noriyuki et al. (2000), pointed out that coffee may inhibit the elevation of serum AST and/or ALT levels and this effect may be more pronounced in the protection of the development of a higher serum AST/or ALT levels. Coffee consumption, has been associated with reduced serum levels of amino transferases ALT (Ruhl & Everhart, 2005; Klatsky et al., 2006; Ikeda et al.,2010; Yamashita et al., 2012)and aspartate amino transferences (Klatsky et al., 2006; Ikeda et al., 2010; Jang et al.,2012) as well as alkaline Phosphatase (ALP) (Casiglia et al., 1993). ALT levels have been considered a more specific marker of liver injury. In this study, ALT was significantly ($p<0.001$) increased in NS-LD and CBC-treated rats, while ALP was significantly ($p<0.001$) reduced. Some animal studies have indicated that caffeine is capable of protecting against toxin induced liver damage (Shim et al., 2013) while other studies suggest that coffee compounds other than caffeine may offer similar benefits (Salazar-Martinez et al., 2004). Several studies have examined the effect of coffee consumption on serum levels of ALT: (Casiglia et al., 1993; Ruhl & Everhart, 2005; Yamashita et al., 2012; Klatsky et al., 2006). Klatsky et al., (2006) found to decrease levels of ALT in human studies who consumed more cups of coffee compared with the non-coffee drinker. Some other studies on AST (Yamashita et al., 2012; Ikeda et al., 2010) and ALP (Casiglia et al., 1993) reported an association of heavier consumption of coffee with lower levels of liver enzymes.

NSC treated groups decreased ($p<0.001$) serum sodium levels compared with control, while CBC treated groups increased the levels of serum sodium suggesting a brand effect. Physiologically, increased sodium levels usually indicate primarily a lack of water and decrease sodium levels indicate an excess of water in the plasma rather than changes in sodium balance, although decrease sodium levels are common after salt lost because of excessive arginine vasopressin (AVP) stimulating water retention. Dehydration increases sodium levels by decreasing its dilution. This occurs when water is lost from the ECF component in excess of sodium (Mattson, 1999). Most biochemical reactions in the body depend upon water and electrolyte balance which are important for maintaining life, physical and mental performance.

NSC treated groups increased serum potassium levels while CBC treated group decreases the levels suggesting a brand effect. Several factors influence the distribution of potassium between cells and extracellular fluid. Dietary deficiency (or excess) is rarely a primary cause of hypokalemia (or hyperkalemia). However, with a pre-existing condition, dietary deficiency (or excess) can enhance the degree of hypokalemia (or hyperkalemia). Extracellular osmolality and acid-base balance also influence transcellular K+ exchange by means of either the Na+/k+ATPase pump or ion channels in the cell membrane. An acute increase in the serum osmolality cause K+ to move out of the cells (Porth & Kunert, 2000).

Reduction in plasma bicarbonate may reflect metabolic acidosis and an abnormal rise in bicarbonate may indicate acute or chronic alkalosis (Edward et al., 2005). It has been shown that alkalosis and acidosis involve a primary or initiating event and a compensatory state that result from the homeostatic mechanism that attempts to correct or prevent a large change in pH. These compensatory mechanisms provide means of control pH when the correction has been impossible or cannot be immediately achieved (Porth & Kunert, 2002).

The plasma [Cl-] concentrations have an inverse relationship with which the filtered [HCO3-] reabsorption. Although the mechanism of this reciprocal relationship is not properly understood, one explanation may be the existence of the well-known chloride shift in response to changes in bicarbonate ion to maintain the electrical neutrality between plasma and cell compartment. As a consequence of the reciprocal relationship as plasma bicarbonate [HCO3-] levels rise, chloride in the plasma falls. This inverse relation serves to maintain the sum of the plasma concentration of [HCO3-] and chloride [Cl-] constant (Nelson & Cox, 2000).

In conclusion, CBC at doses given reduced weight TC, TG, LDL, VLDL, and AST while Nescafe 3 in 1TM increased ALT, urea, K+, reduced TG, LDL, VLDL, Na+, Cl- in the rat. Further investigations are needed on these brands of coffee.

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