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Bioassay of male albino rats treated with cocoa (*Theobroma cacao* Linn)

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ABSTRACT

This paper is aimed at evaluating the activities of cocoa on male rats. Sixty healthy matured rats weighing 130±20g were divided into four experimental groups of 15 rats each. The rats were treated with cocoa seed powder at 0 (control), 100, 200 and 300mg/kg body weight, respectively for 65 days. Blood samples were collected for hormonal, biochemical and haematological indices while semen was obtained for semen analysis. Results showed that cocoa powder elevated prolactin, testosterone and luteinizing hormone (LH) levels; red blood cell, and white blood cell counts significantly reduced follicle stimulating hormone (FSH), estradiol; platelets, packed cell volume and haemoglobin concentration (Hb); alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin. There was also a reducing effect of cocoa powder on total cholesterol, triglycerides, LDL- cholesterol and HDL-cholesterol, especially at the dose of 300mg/kg as well as sperm count, sperm viability and sperm motility. However, sperm head abnormality increased in a dose-dependent manner. These results imply implicitly that cocoa powder at higher doses might be detrimental to animal health, its beneficial effects notwithstanding.

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Introduction

The need to broaden raw material base of agro-allied industries coupled with the paradigm shift from the use of synthetic drugs in medications have led to the search for food/cash crops that will be multifaceted in their derivable benefits (Ikpeme *et al*., 2012a). This according to Ikpeme *et al.* (2012b) will undoubtedly offer great array of opportunities for the sustainability of cultivation and productivity of the crops.

Cocoa (*Theobroma cacao* Linn) which belongs to the family of Sterculiaceae is grown throughout the tropics (Coe, 2000) has been a major cash crop that has immensely contributed to the foreign earnings of Nigeria economy. West Africa produces more than two-third of the World's cocoa with Cote d'Ivorie and Ghana alone accounting for 40 and 20% of the World production, respectively (Anon 2005). According to Dillinger et al. (2000) medicinal uses of cocoa had been traced from Mexican sources and approximately 150 uses of cocoa for medical treatment had been documented. Cocoa contains various bioactive compounds, which includes polyphenols, peptides, minerals and methylxanthines (caffeine, theobromine and theophylline). Cocoa bean and its products are food sources rich in phenolic compounds. Dreosti (2000) reported that 60% of the total phenolics in raw cocoa beans are flavonol monomers (epicatechin and catechin) and procyanidin oligomers (dimer to decamer). Studies had demonstrated that the consumption of cocoa or chocolate reduced the risk of cardiovascular disease (Keen *et al.* 2001).

Interestingly, there are lots of reports that have x-rayed the therapeutic relevance of cocoa and its products even using human subjects (Baba *et al*., 2000; Rein *et al.*, 2000; Wang *et al.*, 2000; Ying *et al.*, 2001; Zhu *et al.*, 2002; Muthur *et al.*, 2002; Murphy *et al.*, 2003; Mursu *et al.*, 2004; Osakabe *et al.*, 2004; Heiss *et al.*, 2005; Taubert *et al.*, 2007; Cooper *et al.*, 2008).

This notwithstanding, there are still fears that cocoa might elicit negative effects on the physiological and biochemical processes of an organism's system (Abrokwah *et al.*, 2009). This is predicated upon the following: (a) cocoa contains diverse bioactive compounds whose synergism might be detrimental to animal health (Greer *et al.*, 2001; Rios *et al.*, 2003); (b) cocoa processing methods differ and this obviously might influence the level of bioactive components therein (Wollgast and Anklam, 2000; Kyi *et al.*, 2005; Caligiani *et al.*, 2007; Abbe and Amin, 2008) and (c) cocoa from different origin and varieties contains different bioactive constituents, which might elicit diverse pharmacological actions (Caligiani *et al.*, 2007; Azizah *et al.*, 2007).

Going by this understanding therefore it becomes imperative to assess the effect of unfermented sun-dried cocoa on male albino rats as regards sperm quality, blood, hormonal and biochemical parameters. This will immensely help in creating awareness, especially to the local cocoa farmers who consume it, the form notwithstanding.

Materials and Method

Preparation of test material

Matured and ripe cocoa pods (Amelonado) were collected from an old cocoa plantation belonging to Cocoa Research Institute of Nigeria, Ibadan (7°14 N, 3°51E), courtesy of Dr. Peter Aikpokpodion. The pods were broken with wooden stick and the beans were wrapped with polyethylene nets and inserted into a heap of cocoa beans to be fermented for six days. This was done so that enough heat will be provided for the experimental beans. At the end of the fermentation period, the tied polyethylenes net with its content were brought out of the

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heap of cocoa and sun-dried for seven days under intense sunlight and pulverized.

Experimental animals

Sixty healthy matured male albino rats weighing between 120- 150 grams were used in the present study. They were housed in cages under standard laboratory conditions of temperature range of 25-29˚C and 12h light/dark cycle throughout the experimental periods. The rats were left to acclimatize for two weeks with water and feed *ad libitum*. Four experimental groups of fifteen rats each per treatment were used for the study using completely randomized design. The rats were treated with cocoa powder at 0 (control), 100, 200 and 300mg.kg body weight, respectively for 65 days. The cocoa powder was mixed with about 10-30% of the daily feed consumption and given in the morning, to ensure the consumption of the daily treatment dose before the remaining feed was given later in the afternoon.

Collection of blood and preparation of serum samples

Blood samples from the rats in the different treatment groups and controls were collected through cardiac puncture after chloroform anaesthesia into serum separator tubes. The blood was allowed to clot by standing at room temperature for 1 hr then refrigerated for another 1 hr. The resulting clear supernatant was spun at 2500-3000 rpm for 10min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-25˚C, which yielded the serum. This was stored at -30ºC until used (Gatsing *et al*., 2005).

Lipid profile analysis

Serum triglycerides were estimated according to the methods of Stein and Myers (1995) while the determination of serum cholesterols was done according to methods of Braun (1984). Serum HDL-cholesterols was estimated according to the methods of Hiller (1987) while serum LDL-cholesterols was estimated according to the methods of NCEP (1988).

Hormonal assay

Serum samples were assayed for levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone/ interstitial cell stimulating hormone (LH/ICSH), estrogen (estradiol) and prolactin using the Microwell enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc., USA) (Ekaluo *et al*., 2010).

Biochemical assays

These assays were adapted from Edet *et al.* (2011). The analysis for Alkaline phosphatase activity in the diluted sample was determined by an optimized and standardized colorimetric (Randox kit) method according to the recommendation of the German Society of Clinical Chemists (GSCC). The absorbance was measured using Optima Spectrophotometer SP-300 (Optima Inc. Chicago, USA). Alanine and aspartate aminotransferases' activities were also determined using analytical kits obtained from Randox.

Estimation of haematological parameters

Blood samples from the different groups were diluted to 1:200 with Hayem's fluid for preservation of the corpuscles. The method of Decie and Lewis (2001) was adopted in the estimation of red blood cell count while white blood cell count was done according to the methods of Cheesbrough (2000). The method (using Sahli's haemoglobinometer) was employed for estimation of hemoglobin (Hb) content of the blood while packed cell volume (PCV) was done using the macrohaematocrit.

Sperm quality analysis

Estimation of sperm count

This was carried out according to the method of Ekaluo *et al.* (2009). The epididymal content was obtained by macerating with fine scissors known weights of the caput and cauda epididymes in a glass petridish containing physiological saline in the ratio of 1:10w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. The sperm cells were counted by cytometry. Five different counts were done for each sample, and the mean were taken as the mean count for each male rat.

Evaluation of sperm motility

The sperm cell suspension was diluted in 2ml of physiological saline and dropped on glass slides. This was viewed under light microscope as to determine the motile and non – motile sperm cells by their movement (WHO, 1992).

Sperm viability determination

This was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin – nigrosin stain and air dried smears were prepared on glass slides for each samples according to Bjorndahl *et al.* (2003). The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin – nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiplied by 100.

Sperm head abnormality test

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al.* (2009).

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Predictive Analytics SoftWare (PASW), version 18.0.

Results

Effect of cocoa on reproductive Hormones

The effect of administering cocoa powder on male rats showed that there were significant ($P < 0.05$) differences between the rats in the control and the treated rats, which tends to be dose-dependent. Our result revealed that the levels of prolactin, testosterone and Luteinizing hormone (LH) increased steadily with increasing dose while the level of estradiol increased more when the rats were administered with 200mg/kg of the cocoa powder (Table 1).

Effect of cocoa on haematological parameters

Except for the red blood cell and white blood cell counts, administering rats with cocoa powder did not significantly ($P > 0.05$) affect the platelets, packed cell volume and haemoglobin concentration. However, though there were significant differences between the treated rats and those in the control, among the treated groups, there were no significant differences (Table 2).

Effect of cocoa on liver enzymes

Cocoa powder showed a reducing effect on the levels of ALT, AST, ALP and albumin while it did not show any

significant effect on total protein. Additionally, when 100mg/kg of the powder was administered to the rats, there was an upsurge on the levels of AST, ALP and albumin (Table 3).

Effect of cocoa on lipid profile

There were initial increase in the levels of total cholesterol and high density lipoprotein (HDL) while there was no difference between triglyceride and low density lipoprotein levels when rats were treated with 100mg/kg and 200mg/kg, respectively. Generally, in all the lipid parameters studied, their levels reduced drastically when the rats were treated with 300mg/kg of cocoa powder.

Effect of cocoa on sperm quality and quantity

There was significant effect ($P < 0.05$) of cocoa treatment on sperm quality and quantity. The treatment caused significant decrease in the sperm counts, sperm viability, sperm motility while sperm head abnormality increased with increasing concentration of the powder.

Discussion

The economic and therapeutic benefits derivable from cocoa and its products notwithstanding, it is important to appreciate the fact that the pharmacological potency of single isolated compound differs from the effect when all the bioactive constituents of any particular plant synergistically interact to deliver their medicinal effects. It is already established that cocoa is rich in polyphenols (Arts *et al.*, 2000; Natsume *et al.*, 2002; Cooper *et al.*, 2007; Aikpokpodion and Dongo, 2010), Greer *et al.* (2001), Rios *et al.* (2003) also reported that cocoa is rich in methylxanthines, which includes caffeine, theobromine and theophylline and these might posses both positive and negative health effects.

Our result on hormonal profile revealed that administering cocoa powder to male rats has significant boosting effect on prolactin, testosterone and luteinizing hormones (LH), respectively with increasing dose while follicle stimulating hormone (FSH) level was more affected at the dose of 200mg/kg. The implication of the above result is that cocoa powder might be hormone-specific in its action. Given that cocoa contains various bioactive compounds including minerals, it is probable that these different bioactive components might either singly or synergistically elicit hormone specific action. Though our present report suggests that cocoa powder boosted the level of testosterone, it was not significant enough to enhance the production of sperm cells, leading to the reduction of sperm counts. It should be understood that testosterone does not function alone as it needs other hormones such as LH and FSH to perfectly carry out its function. According to Wang et al. (1992) theobromine intake caused vacuolation within the Sertoli cells, altered spermatid shape, and failure in the release of late spermatids in male rats. Similarly, Soffietti *et al*. (1989) observed that high dose of cocoa extract containing theobromine could alter testis structure. It is therefore very likely that the effects of cocoa powder on sperm parameters are not unconnected with the distortion on the cytoarchitecture of the rat's gonads (Nwanjo *et al.,* 2007; Ikpeme *et al.*, 2012a). It presupposes that when the integrity of cyto-architecture of the gonads is compromised, spermatogenic processes and pathways are also compromised with the concomitant drastic reduction in the quality and quantity of sperm cells.

Interesting to mention is the fact that cocoa contains the same xanthine stimulants like caffeine, theobromine and theophylline (Odebunmi *et al.*, 2009) and has been reported to mediate some physiological effects that are in tandem with the actions of refined caffeine (Corrillo and Bennitez, 2000; Dash and Gummadi, 2008). Caffeine interacts chemically with rapidly replicating DNA in growing cells, thus inducing mutations, though there are controversies regarding the genotoxic and mutagenic effects of caffeine in biological systems (Utulu and Bakare, 2010). Undoubtedly, exposure of the seminal fluid to chemicals (toxic) could result to functional or structural impairment of sperm cells, which obviously could be linked to the alterations in testicular DNA, thus disrupting the process of differentiation of spermatozoa. Since the pituitary-hypothalamic axis control spermatogenesis, it is possible that the bioactive constituents inherent in cocoa powder might have produced debilitating effects on this axis.

Cocoa intake decreased low density lipoprotein (LDL) [Mathur *et al.,* 2002] while Wang et al. (2001) demonstrated that cocoa powder and dark chocolate supplementation improved high density lipoprotein (HDL) level by 4% compared to control diet. Allen *et al.* (2008) in a similar experiment reported that dark chocolate consumption, especially containing 180mg polyphenols reduced total and LDL cholesterol among elevated serum cholesterol subjects. Interestingly, our present result of lipid profile agrees with these earlier submissions. Fermentation of cocoa seeds reduces the polyphenolic compounds. Caligiani et al. (2007) submitted that (-) –epicatechin and (+)-catechin) (forms of polyphenols) are highest in an unfermented cocoa seeds. Understandably, the percentage of the polyphenolic compounds in the cocoa seeds is of less import than their bioavailability in the organism's system. According to Scalbert and Williamson (2000), Donovan *et al.* (2006) the bioavaibility of polyphenols depends to a large extent on the chemical structure, glycosylation, acylation, conjugation and polymerization. Additionally, it is also influenced by the protein factor (Serafini *et al.,* 2003; Tabernero *et al.*, 2006), which might not necessarily be salivary protein but also with dietary protein and digestive enzymes and this might influence their transportation and absorption activities (Brunet *et al.,* 2002). This however, suggests that the polyphenolic compounds in the cocoa powder were bio-available to the recipient organs leading to the effect observed in our report.

Any herbal therapy that has the potential to protect against artherosclerosis and its associated or incidental cardiovascular complications will be a welcomed venture. Though there are initial increase in total cholesterol and HDL; increasing the dose of cocoa powder to 300mg.kg reduced them drastically. Unfortunately, the reduction in HDL level could spell doom to the system, the reduction in total cholesterol notwithstanding. Mathur *et al.* (2002), Baba *et al.* (2007) documented the protective effect of cocoa polyphenols towards LDL oxidation, which is an early indicator for the development of cardiovascular diseases. Earlier reports showed that there was an elevation of HDL-cholesterol which was not in tandem with our current report. It is probable that the variants of the polyphenolic compounds inherent in the cocoa powder orchestrated by the origin and variety of the cocoa (Clapperton *et al.,* 1994; Caligiani *et al*., 2007; Azizah *et al.,* 2007) will partly give an explanation to the differential.

The integrity of the liver structurally and functionally can be predicated on the levels of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), albumin, etc. According to MacSween and Whaley (1992) elevation of ALT might suggest the presence of viral hepatitis, congestive heart failure, liver

damage while Varley et al. (1991) observed that albumin reduction is usually associated with chronic liver disease, cirrhosis as well as nephritis. It might be erroneous to submit that since albumin level decreased at higher doses of cocoa powder, it could be an early indication of liver damage. Reduction in ALT level should have disabused minds concerning a seeming liver problem. These notwithstanding, it will be wise to mention here that these enzymes are not all it takes for the liver to function perfectly there are other intrinsic factors.

The administration of cocoa powder to male rats did not significantly affected the platelets, packed cell volume (PCV) and haemoglobin concentration (Hb). Terashima *et al.* (2002) reported that flavonoids as antioxidant maintains the haeme iron in its form and thus enhance erythropoiesis. This was also observed by Jaja *et al.* (2002), Ahumibe and Braide (2009) suggesting that there exist a relationship between antioxidant activity and haemoglobin quality as ascorbic acid increases haemoglobin level significantly. It is as well obvious that cocoa and its products contain very high antioxidant bioactive components, especially polyphenols and flavonoids. Esomonu *et al.* (2005) observed that the formation of complexes between flavonoids and minerals might affect haemoglobin synthesis and erythropoiesis. It is probable that the protein factor might have complexed with polyphenols making the bioavailability of polyphenols difficult (Brunnet *et al.*, 2002; Serafini *et al.*, 2003; Tabernero *et al.,* 2006) to the target cells, thus reducing their anti-oxidative potential. Implicitly, the protein state of the rats may have contributed to the reducing efficacy of the polyphenol in cocoa in exerting antioxidant properties. It is still obvious that though all polyphenols possess antioxidant properties *in vitro* but are not likely to exert the same properties *in vivo* (Baba *et al.,* 2000).

It might be erroneous at this juncture to attribute the effects of the cocoa seed powder on the parameters studied to single bioactive compound rather synergistic interactions among these bioactive compounds.

Conclusion

Implicitly, however, cocoa seed powder administration to male rats might not be advisable giving the reducing effects it has on sperm and haematological parameters, respectively. It does therefore suggest that its use as an antioxidant should be done with caution as a safe drug should be safe holistically.

References

Abbe Maleyki, M.J. and Amin, I (2008). Polyphenols in cocoa and cocoa products: Is there a link between antioxidant properties and health. Molecules 13: 2190-2219

Abrokwah, F. A., K. A Asamoah, , and Esubonteng, P. K., (2009). Effects of the intake of Natural Cocoa powder on some biochemical and haematological indices in the rat. *Ghana medical journal 43* : 164-168.

Ahumibe A. A. and Braide V. B., (2009). Effect of gavage treatment with pulverised Garcinia kola seeds on erythrocyte membrane integrity and selected haematological indices in male albino wistar rats. *Nig. J. Physiol. Sci.* 24 (1): 47-52.

Aikpokpodion, P. A and Dongo, L. N (2010). Effects of fermentation intensity on polyphenols and antioxidant capacity of cocoa beans. ***Int. J. Sustain. Crop Prod. 5(4):66-70***

Allen, R.R., Carson, L.A., Kwik-Uribe, C., Evans, E.M., and Erdman, J.W. (2008) Daily consumption of a dark chocolate containing flavanols and added sterol esters affects cardiovascular risk factors in a normotensive population with elevated cholesterol. *J. Nutr. 138*, 725-731.

Anon (2005) Annual Report 2003/2004. International Cocoa Organization,www. icco. org.

Arts, I.C.W., van de Putte, B., Hollman, P.C.H. (2000). Catechin contents of foods commonly consumed in the Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J. Agric. Food Chem. 48*, 1746-1751.

Azizah, O.; Amin, I.; Nawalyah, A.G.; Ilham, A. (2007). Antioxidant capacity and phenolic content of cocoa beans. *Food Chem.*, *100*, 1523-1530.

Baba, S., Natsume, M., Yasuda, A., Nakamura, Y., Tamura, T., Osakabe, N., Kanegae, M., and Kondo, K. (2007) Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo and hypercholesterolemic humans after intake of different levels of cocoa powder. *J. Nutr. 137*, 1436–1441.

Baba, S., Osakabe, N., Kato, Y., Natsume, M., Yasuda, A., Kido, T., Fukuda, K., Muto, Y., and Kondo, K. (2007). Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. *Am. J. Clin. Nutr*., *85*, 709-717.

Baba, S., Osakabe, N., Natsume, M., Yasuda, A., Takizawa, T., Nakamura, T.and Terao, J. (2000). Cocoa powder enhances the level of antioxidative activity in rat plasma. *Bri. J. Nutr. 84*, 673-680.

Brunet, M. J., Blade, C., Salvado, M. J. and Arola, L. (2002). Human Apo A-I and rat transferring are the principal plasma proteins that bind wine catechins. *Journal of Agriculture and Food Chemistry,* 50:2708-2712.

Bjórndahl, L., Sódourlund, I., and Kvist, U. (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Human Reproduction. 18*:813-816.

Caligiani, A., Cirlini, M., Palla, G., Ravaglia, R., and Arlorio, M. (2007). GC-MS detection of chiral markers in cocoa beans of different quality and geographic origin. *Chirality*, *19*, 329-334.

Cheesbrough, M., (2000). *District Laboratory Practice in Tropical Countries.* Cambridge: University Press. Pp. 45-50

Clapperton, J., Lockwood, R., Romanczyk, L.,and Hammerstone, J. F. (1994) Contribution of genotype to cocoa (*Theobroma cacao* L.) flavour. *Trop. Agric. (Trinidad) 71*, 303-308.

Coe, S. D. (2000). *True history of chocolate.* Thames and Hudson Publishers: New York. composition during the manufacture of chocolate and methodology for identification and quantification. *Food Res. Int. 33*, 423-447.

Cooper, K.A., Campos-Gimenez, E., Alvarez, D.J., Nagy, K., Donovan, J.L., and Williamson, G. (2007). Rapid reverse-phase ultra-performance liquid chromatography analysis of the major cocoa polyphenols and inter-relationship of their concentration in chocolate. *J. Agric. Food Chem.*, *55*, 2841-2847.

Cooper, K.A.; Donovan, J.L.; Waterhouse, A.L.; Williamson, G. (2008) Cocoa and health: a decade of research. *Bri. J. Nutr. 99*, 1-11.

Corrillo, J A. and Bennitez, J. (2000). Clinically significant pharmacokinetic interactions between dietary caffeine and medication. Clinical Pharmacokinetics 39(12): 127- 153.

Dacie, J. V and Lewis, S. M., (2001). *Practical Haematology. (*11th ed) Hong Kong: Longman Group.Ltd. pp. 11-17

Dash, S. S and Gummadi, S. N (2008). Inhibitory effect of caffeine on growth of various bacterial strains. Research Journal of Microbiology 3:457-465.

Table 1: Hormonal parameters of rats administered with cocoa Seed powder

Hormonal Parameters	Concentration (mg/kg BW)			
	0	100	200	300
F S H (Miu/ml)	1.4^{b}±0.16	0.9^{a}±0.04	1.5^{b}±0.11	1.35^{b}±0.18
Estradiol (Pg/ml)	0.16^{a}±0.01	0.27^{b}±0.08	0.32^{c}±0.012	0.29^{b}±0.016
Prolactin (ng/ml)	11.1^{a}±0.48	12.9^{b}±0.31	15.32^{c} ± 0.46	18.72^{d} ±0.23
Testosterone (ng/ml)	5.35^{a}±0.25	5.28^{a}±0.37	7.23^{b}0.11	9.33^{c}±0.12
LH(miu/ml)	8.27^{a}±0.27	13.6^{b}±0.45	13.95^{b}±0.29	15.15^{c}±0.09

*Means followed by the same case letter along horizontal arrays indicate no significant difference (P<0.05).

Table 2: Haematological parameters of rats administered with cocoa seed powder

Haematological Parameters	Concentration in (mg/kg BW)			
	0	100	200	300
RBC (10^9/L)	7.82^{a}+0.29	9.68^{b}±0.21	9.41^{b}±0.37	9.29^{b}±0.44
Platelets	605.29^{a}±17.14	663.25^{a}±35.56	608.32^{a}±12.25	640.5^{a}±18.18
PCV (%)	45.0^{a}±1.47	48.75^{a}±1.11	49.0^{a}±2.38	46.75^{a}±2.69
Hb conc. (g/dL)	16.32^{a}±0.76	16.03^{a}±0.44	16.2^{a}±0.48	17.03^{a}±0.45
WBC (10^9/L)	6.08^{a}±0.22	11.1^{b}±0.16	11.75^{b}±0.40	6.13^{a}±0.55

*Means followed by the same case letter along horizontal arrays indicate no significant difference (P<0.05)

Table 3: Effect of cocoa powder on some liver enzymes

Hormonal Parameters	Concentration (mg/kg BW)			
	0	100	200	300
Alanine aminotransferase	31.58^{a}±0.15	25.69^{a}±0.94	54.14^{b}±8.00	35.11^{a}±4.10
Aspartate aminotransferase	50.57^{a}±6.21	125.69^{b}±9.29	105.17^{b}±0.012	61.44^{a}±9.15
Alkaline phosphatase	303.91^{d}±0.59	167.26^{c}±0.38	81.47b ± 0.28	45.24^{a} ±0.51
Total protein	6.05^{a}±0.03	6.72^{a}±0.06	6.83^{a}± 0.11	7.58^{c}±0.45
Albumin	31.57^{a}±0.16	47.33^{b}±3.86	32.27^{a}±0.53	35.53^{c}±1.40

Means followed by the same case letter along horizontal arrays indicate no significant difference (P<0.05)

Table 4: Effect of cocoa powder on some lipid profile

Hormonal Parameters	Concentration (mg/kg BW)			
	0	100	200	300
Serum triglyceride (mg/dl)	86.84^{a}±0.18	149.69^{b}±2.74	134.45^{b}±3.23	75.93^{a}±1.81
Total cholesterol (mg/dl)	62.47^{a}±0.19	95.35^{b}±9.29	62.84^{a}±3.12	75.90^{a}±4.21
HDL-cholesterol (mg/dl)	71.06^{a}±0.06	79.40^{b}±0.38	70.23^{a} ± 0.34	72.45^{a} ±0.29
LDL-cholesterol (mg/dl)	17.66^{a}±0.15	30.26^{b}±0.06	26.58^{a}± 3.36	15.11^{c}±0.33

Means followed by the same case letter along horizontal arrays indicate no significant difference (P<0.05).

Table 5: Effect of cocoa powder on sperm quality and quantity

Hormonal Parameters	Concentration (mg/kg BW)			
	0	100	200	300
Sperm count (x10^6/ml)	6.75^{b}±0.08	6.55^{b}±2.74	6.05^{a}±0.12	5.96^{a}±0.13
Sperm head abnormality (%)	4.99^{a}±0.58	5.77^{b}±1.25	8.47^{d}±1.84	8.20^{c}±1.64
Sperm viability (%)	89.7^{d}±1.18	84.79^{c}±0.38	79.6^{b} ± 0.24	76.87^{a} ±0.18
Sperm motility (%)	83.30^{d}±0.17	52.5^{a}±0.12	55.00^{b}± 1.15	57.50^{c}±0.21

Means followed by the same case letter along horizontal arrays indicate no significant difference (P<0.05).

Dillinger, T.L., Barriga, P., Escarcega, S., Jimenez, M., Lowe, D.S. and Grivetti, L.E. (2000). Food of the Gods: Cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J. Nutr. 130*, 2057S-2072S.

Donovan, J.L., Crespy, V., Oliveira, M., Cooper, K.A., Gibson, B.B. and Williamson, G. (2006). (+)- Catechin is more bioavailable than (-)-catechin: Relevance to the bioavailability of catechin from cocoa. *Free Rad. Res. 40*, 1029-1034.

Dreosti, I.E.(200). Antioxidant polyphenols in tea, cocoa, and wine. *Nutrition*, *16*, 692-694.

Edet, E. E., I.J. Atangwho, M.I. Akpanabiatu, T. E. Edet, F.E. Uboh, E. David-Oku. (2011). Effect of *Gongronema latifolium* Leaf Extract on some Liver Enzymes and Protein Levels in Diabetic and non Diabetic Rats. *J. Pharm. Biomed. Sci.*, 1(5) 104-107.

Ekaluo, U.B., Ikpeme, E.V. & Udokpoh, A.E. (2009). Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. The Internet Journal of Toxicology, 7(1).

Ekaluo, UB., Ikpeme, EV., Udensi, O., Markson, AA., Madunagu, BE., Omoun, G. and Umana, E. J. (2010). Effect of aqueous leaf extract of neem (*Azadirachta indica*) on the hormonal milieu of male rats. International Journal of Current Research, 4: 1-3

Esomonu, U.G., A.B. El-Taalu, J.A. Anuka, N.D. Ndodo, M.A. Salim and M.K. Atiku., (2005) . Effect of ingestion of ethanol extract of *G. kola* seeds on erythrocytes in wistar rats. *Nig. J. Physiol. Sci.,* 20:30-32.

Gatsing, D., Aliyu, R., Kuiate, J. R., Garba, I.H., Jaryum, K. H., Tedongmo, N., Tchouanguep, M. F. and Adoga, G. I. (2005).

Toxicological evaluation of the aqueous extract of *Allium sativum* bulbs on laboratory mice and rats. Cameroon Journal of Experimental Biology, 01:39-45.

Greer, F.. Hudson, R., Ross, R., and Graham, T. (2001). Caffeine ingestion decrease glucose disposal during a hyperinsulinemic-euglycemic clamp in sedentary humans. *Diabetes*, *50*, 2349-2354.

Heiss, C., Kleinbongard, P., Dejam, A., Perre, S., Schroeter, H., Sies, H., and Kelm, M. (2005). Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J. Am. Coll. Cardiol.*, *46*, 1276-1283.

Hiller, A. (1987). National Cholesterol education programme. Recommendations for HDL-cholesterol measurements. Clin. Chem., 33:895.

Ikpeme, E. V., A. I. Nta, U. B. Ekaluo and O. Udensi (2012a). Phytochemical Screening and Haematological Evaluation of *Parkia biglobossa* and *Gongronema latifolium*. *J. Basic. Appl. Sci. Res.*, 2(3): 2599-2606.

Ikpeme, E. V., O. Udensi, U. B. Ekaluo and E. A. Uyoh (2012b). Ethnobotanical relevance of spice plants [*Parkia biglobossa* and *Gongronema latifolium*]: Indices for conservation and domestication. *J. Basic. Appl. Sci. Res.*, 2(3): 3094-3102.

Jaja, S. I., Ikotun, A. R., Gbenebitse, S. and Temiye, E. O., (2002). Blood pressure, haematologic and erythrocyte fragility changes in children suffering from sickle cell anaemia following ascorbic acid supplementation. *J. Trop. Paed*. 48: 366-370

Keen, C. L, Holt, R. R, Polagruto, J. A, Wang J. F, and Schmitz, H. H. (2001). Cocoa flavanols and cardiovascular health. Phytochemistry Reviews 1, 231-240.

Kyi, T.M., Wan Ramli, W.D., Abu Bakar, M., Mohd. Wahid, S., Abdul Amir, H.K., and Meor Zainal, M.T. (2005). The kinetics of polyphenol degradation during the drying of Malaysian cocoa beans. *Int. J Food Sci. Technol. 40*, 323-331.

MacSween, R. M. N. and Whaley, K. (1992). Muir's textbook of pathology. New Deihi: ELBS Publishers.

Mathur, S., Devaraj, S., Grundy, S.M., and Jialal, I. (2002). Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J. Nutr. 132*, 3663-3667.

Murphy, K.J., Chronopoulos, A.K., Singh, I., Francis, M.A., Moriarty, H., Pike, M.J., Turner, A.H., Mann, N.J., and Sinclair, A.J. (2003). Dietary flavanols and procyanidin oligomers from cocoa (*Theobroma cacao*) inhibit platelet function. *Am. J. Clin Nutr.*, *77*, 1466-1473.

Mursu, J., Voutilainen, S., Nurmi, T., Rissanez, T.H., Virtanen, J.K., Kaikkonen, J., Nyyssonen, K., and Salonen, J.T. (2004). Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. *Free Rad. Biol. Med. 37*, 1351–1359.

Natsume, M. Osakabe, N., Takizawa, T., Nakamura, T., Miyatake, H., Hatano, T. and Yoshida, T. (2002). Analysis of polyphenol constituents in cocoa and chocolate. In *Quality Management of Nutraceuticals*; Ho. C-T.; Zeng, Q.Y., Eds.; ACS Symposium Series 803; American Chemical Society: Washington, DC, USA, pp. 180-198.

NCEP (National Cholesterol Education Programme) (1988). Expert panel on detection, evaluation and treatment of high blood cholesterol in adult. Arch. Intern. Med., 148:66.

Nwanjo, H.U., Iroagba, I.I., Nnatuanya, I.N., and Eze, N.A. (2007). Antifertility activity of dihydroartemisinin in male albino rats. The Internet Journal of Endocrinology, 4(1)

Odebunmi, E. O., Oluwaniyi, O. O., Awolola, G. V. and Adedeji, D. O. (2009). Proximate and nutritional composition of Kola nut (*Cola nitida*), bitter cola (*Garcinia cola*) and Aliigator pepper (*Afromonmum melegueta*). African Journal of Biotechnology 8(2): 308-310.

Osakabe, N., Baba, S., Yasuda, A., Iwamoto, T., Kamiyama, M., Tokunaga, T., and Kondo, K. (2004). Doseresponse study of daily cocoa intake on the oxidative susceptibility of low-density-lipoprotein. *J. Health Sci. 50*, 679-684.

Rein, D., Lotito, S., Holt, R.R., Keen, C.L., Schmitz, H.H., and Fraga, C.G. (2000) Epicatechin in human plasma: *in vivo* determination and effect of chocolate consumption on plasma oxidation status. *J. Nutr. 130*, 2109S-2114S.

Rios, L.Y., Gonthier, M.P., Remesy, C., Mila, I., Lapierre, C., Lazarus, S.A., Williamson, G., Scalbert, A. (2003). Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am. J. Clin. Nutr. 77*, 912-918.

Serafini, M., Bugianesi, R., Maiani, G., Valtuena, S., De Santis, S. and Crozier, A. (2003). Plasma antioxidants from chocolate. *Nature*, 424:1013.

Scalbert, A. and Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *J. Nutr. 130*, 2073S-2085S.

Soffietti, M.G., Nebbia, C., Valenza, F., Amedeo, S., and Re, G. (1989). Effects of theobromine in mature and immature rabbits. *J. Comp. Pathol. 100*, 47-68.

Stein, E. A and Myers, G. L. (1995). National Cholesterol education programme. Recommendations for triglycerides measurements. Clin. Chem., 41:1421-1426.

Tabernero, M., Serrano, J., and Saura-Calixto, F. (2006). The antioxidant capacity of cocoa products: contribution to the Spanish diet. *Int. J Food Sci. Tech*., *41*, 28-32.

Taubert, D., Roesen, R., Lehmann, C., Jung, N., Schomig, E. (2007) Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide. *JAMA*. *298*, 49-60.

Terashima K, Takaya Y, Niwa M, (2002). Powerful antioxidative agents based on garcinoic acid from *Garcinia kola*. *Bioorg. Med. Chem*. 10:1619-1625.

Utulu, S. and Bakare, A. A. (2010). DNA damage induced in germ and bone marrow cells of mice by caffeine. Research Journal of Biological Sciences, 5: 536-541.

Varley H., Gowenlock A. H., and Bell, M. (1991). *Practical clinical biochemistry*. (15th edition). Delhi: CBS Publishers.

Wang, J.F., Schramm, D.D., Holt, R.R., Ensunsa, J. L., Fraga, C.G., Schmitz, H.H., Keen, C.L. (2000) A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J. Nutr. 130*, 2115S-2119S.

Wang, Y., Waller, D.P., Amiya, P., Hikim, S., Russell, L.D.(1992). Reproductive toxicity of theobromine and cocoa extract in male rats. *Reprod. Toxicol. 6*, 347-353.

World Health Organization (WHO). (1992). Laboratory manual for the examination of human semen and sperm cervical mucus interaction. Cambridge University Press, Cambridge.

Wollgast, J. and Anklam, E. (2000). Review on polyphenols in *Theobroma* cacao: Changes in the rat. *Ghana medical journal 43* : 164-168.

Ying Wan, Joe A Vinson, Terry D Etherton, John Proch, Sheryl A Lazarus, and Penny M Kris- Etherton (2001) Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am. J. Clin. Nutr.*, *74*, 596-602.

Zhu, Q.Y.; Holt, R.R.; Lazarus, S.A.; Orozco, T.J.; Keen, C.L. (2002). Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. *Exp. Biol. Med. 227*, 321-329.