

Greener Journal of Medical Sciences

ISSN: 2276-7797 ICV: 5.98

Submitted: 20/05/2016

Accepted: 29/05/2016

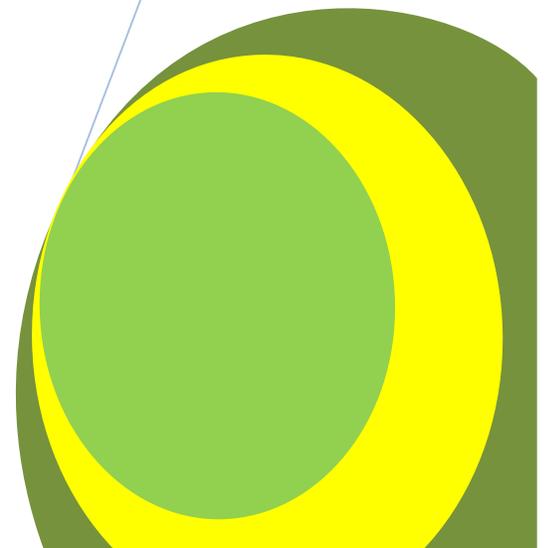
Published: 25/06/2016

DOI: <http://doi.org/10.15580/GJMS.2016.3.052016094>

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Research Article (DOI: <http://doi.org/10.15580/GJMS.2016.3.052016094>)

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ABSTRACT

The activities of Coffee on the fertility hormones, haemostatic and biochemical profile of female Albino wistar rats have been investigated. The rats (180 -200g) were housed in the animal house of the Dept. of physiology of the University. The rats were divided into two groups of 12 rats(control)and24 rats [Test].Group 1[control]was fed with normal rat feed and tap water ad libitum while group 2(Test rats)were given rat feed Coffee extract and tap water ad libitum. The study lasted for 28days.At the end of the experiment, cardiac puncture was used to collect blood samples for hormonal assay, and blood analysis. Results showed that fertility hormones (FSH, LH, Prolactin, Progesterone, Testosterone) and haematological indices in the test animals were significantly reduced ($p<0.05$) compared to the control rats. The extract also prolonged the bleeding time, clotting time, Prothrombin time and Partial thromboplastin kaolin test ($p<0.05$), but most of the liver function profile were not significantly affected except for alkaline phosphatase which was reduced. Therefore it is concluded that Coffee extract affects fertility hormones in rats but does not affect the liver function profiles and protein level, hence suggesting a continuum of favourable effect on liver function and protein concentration of the body.

Keywords: Caffeine, fertility hormones, bleeding time, Prothrombin time, Liver enzymes, haemostasis, Haemoglobin.

INTRODUCTION

Every living thing including man requires food for its growth and survival. Food on its own is defined as the science of nutrition which promotes healthy well being. Coffee is a brewed drink with a distinct aroma and flavour. It is prepared from roasted beans which are the seeds found inside "berries" of Coffee plants. Coffee plants are cultivated in over 70 countries primarily in the equatorial regions of America, southeast Asia, India and Africa. The two most commonly grown are the Arabicaand Robusta. Coffee is slightly acidic (ph 5.0-5.1).Coffee rapidly loses some of its flavourafter grinding (Aloy-Amadi Oluchi Chinwe et al 2013).

Haemostasis is a process which arrest blood loss, and whenever a vessel is cut, haemostatic process is activated by successive mechanisms involving vascular spasm, formation of platelet plug, blood coagulation and growth of fibrous tissues into the blood to close the damaged vessel permanently(Arthur,2007). The fertility hormones are hormones responsible for oestrous cycle in rats and ovulation in human female subjects. They are responsible for conception in both animals and humans. The activities of coffee in the fertility hormones, haemostatic and biochemical profile of female albino wistar rats were studied with the curiosity to know the effect of the caffeine content of coffee on follicular stimulating hormone(FSH),Luteinizing hormone(LH), prolactin, progesterone, testosterone, haemoglobin concentrations, Packed cell volumes, white blood cells (wbc), platelet count, Prothrombin time test, bleeding time, clotting time, Partialthromboplastin time kaolin, Totalbilirubin, alkaline phosphatase, a spartate transaminase and alanine transaminase, using female albino wistar rats.

In recent years, it has been observed that there is high consumption of coffee.It has a stimulating effect in humans because of its caffeine content, due to the high caffeine content in coffee, it has been found to have an effect on the fertility of female rats in that it increases the length of time it takes to conceive. This could be because caffeine influences the quality of the developing oocyte.

Health Benefits and Medicinal Values of Coffee:

The positive aspect of coffee drink is that it offers a pleasant means of increasing fluid intake during a workbreak or meal (Aloy-Amadi Oluchi Chinwe 2013). Coffee offers nutritious contributions of potassium and niacin . Coffee

makes one smarter by its caffeine content which blocks the inhibitory effects of adenosine, thus increasing the neuronal firing in the brain and the release of other neurotransmitter like dopamine and epinephrine. Coffee helps to burn fats and improves physical performances due to its stimulant effect on the oxidation of fatty acids. Coffee may drastically lower ones risk of type 11 diabetes, lower ones risk of Alzheimer and Parkinson s diseases, protects ones brain at old age. Coffee lowers the risk of cancer of the liver. It contains lots of nutrients and antioxidants. Coffee increases sleep latency, reduces total sleeping time and improves various motor skills impaired by fatigue.

Chemical Constituents of Coffee

The chemical components of coffee include tannin, caffeine, fibre, water, sugar, protein, fats and oil, vitamins.

MATERIALS AND METHODS

Plant materials:

The coffee beans were obtained by purchasing them from OSE market in Onitsha Anambra state. The seeds were identified by a botanist from botany department of the university where a voucher specimen was deposited at the Botanical store of the university.

Experimental Animals:

Thirty six(36) adult female wistar rats weighing 150-200g were obtained from the animal house of the department of physiology; they were selected randomly and kept in a metal cage with iron netting in the animal house of the department of physiology. They were maintained on standard vital feeds(Guinea feed, Benin)and water ad libitum. They were stabilised for two weeks before being used for the experiment.

Preparation of Coffee Extract:

The coffee beans (Arabica)were washed before placing them in a bucket with 1L of water and left to stand for 18-24hrs to ferment. Later the coffee beans were brought out and spread on a wire drying rack, and put to dry in the sun for five (5)days. The thin tough parchment layer of the beans were removed by rubbing the dried beans on concrete .The beans were roasted in the fire, grounded into fine powder and later,10g of the powder was mixed with 1000ml of sterilised water. The solution was allowed to stand overnight so as to sediment; cheese cloth was used to filter the solution. The solution was stored in a refrigerator in a clean white stoppered bottle.

Phytochemical Analysis of Seed

The presence or absence of alkaloids,flavenoids,resin,saponin,glycosides,tannins,phenols,caffeinoidsterpens, steroids, reducing sugars, acidic compounds, carbohydrates, fats and oils, and various secondary metabolites using standard phytochemical screening procedures as described by Harbourne (1973),Trease and Evans(1996) respectively.

Toxicity study:

The LD₅₀ of the extract in albino wistar rats was determined using Lorcks' method(1983).

Experimental design:

Thirty six female albino wistar rats weighing 150-200g were selected randomly and placed into two groups;Group1 consisting of 12rats are the control group, while group 2 are the test group (24rats)that was given the extract of Arabica (coffee).The control rats were fed normal rat feed and water ad libitum while the test rats were fed the aqueous extract, ratfeed,and water ad libitum daily for 28days.

Administration of extracts to Rats:

The test rats (Group2)were given 5mg/g of the aqueous extract of coffee by inserting the orogastric tube through the mouth to the stomach and then releasing the extract into the tube with the help of a syringe into the stomach daily for 28 days. Finally they were allowed access to their normal rat feed and water ad libitum. The control rats were not given the extracts.

Obtaining of blood sample for tests:

At the end of 28 days of administration of aqueous extract of coffee, the rats were placed under diethyl ether anaesthesia. Bleeding time and clotting times were conducted on the animals; later the neck area was quickly shaved with scissors to expose the jugular veins. (Sambo et al, 2014). The vein after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade and about 3.0 ml of blood sample was collected in E.D.T.A tubes and sodium citrate anticoagulant tubes for haematological and haemostatic tests, and another 5.0 ml of blood also collected into clean sterile tubes which were allowed to clot for 30 mins. This was then centrifuged at 33.5g for 15 mins using a centrifuge. The sera were aspirated with Pasteur pipette and stored frozen until required for the hormonal and biochemical analysis.

Determination of haematological parameters:

Packed cell volume (Pcv), haemoglobin concentration, (Hb), white blood cell count (wbc), and platelet counts were determined using Mindray Haematology Analyzer (Mindray BC-2300. Guangzhou medical Equipment co. Ltd, China).

The Haemostatic Parameters:

The whole blood clotting time was determined using the method of Lee and White (1998), and the Bleeding time was carried out as described by Dejana et al (1982). The Prothrombin time and Partial thromboplastin time kaolin tests were carried out as elaborated in Quick's one stage method (1998).

Determining the Biochemical and Hormonal Assays:

The biochemical assay which is mainly the Liver function tests viz Total bilirubin, conjugated bilirubin, Aspartate transaminase, alanin transaminase, and alkaline phosphatase, and total protein concentration were carried out by the method described by Baker et al, 1998.

The hormonal assay analysis was done with the use of immunoassay kits using the associated serum. This is a tube based enzyme immunoassay (London, U.K and Nigeria). The procedure for the assay as contained in the manufacturer's manual was strictly followed. The within assay variation was 8.19 over the sensitivity which was 0.3 mg/ml for testosterone. The optical density was read using a spectrophotometer (Jenway 6300 spectrophotometer, U.K) that was sensitive at wave lengths between 492 nm and 550 nm.

STATISTICAL ANALYSIS

The data collected were expressed as mean + standard Error of mean. Statistical analysis of variance (ANOVA) and Student's t-test at a level of 5% level of confidence ($p < 0.05$).

RESULTS

Table 1 indicates phytochemical analysis of the bean seed of coffee. The table shows the presence of alkaloids, tannins, caffeine, sugar, calcium, carbohydrates, fat and oil.

The Phytochemical analysis of coffee:

Table 1: Constituents of extracts of Coffee

	Alkaloids	Carbohydrates	Phenols	Tannin	Calcium	Terpenoids	Sugar	Fats and oil	Resins, Acidic cpds, Glycosides
Degree of constitution	+	++	++	++	+	++	++	++	-

Key:

- Negative (absent),
- + Present in small concentrations,
- ++ Present in moderate high amount,
- +++ Present in very high concentrations.

Table 2: Hormonal profiles of the rat fed with coffee extracts for 28 days.

S/N	Hormones(iu/l)	Control n=12	Test Rats n=24,after 28 days coffee extract feeding.
1	FSH iu/ml	250 ± 0.3	1.08 ± 0.2
2	L.H iu/ml	5.1 ± 0.5	3.5 ± 0.4
3	Prolactin ng/ml	5.8 ± 2.5	3.6 ± 1.6
4	Progesterone ng/ml	3.0 ± 0.4	2.0 ± 0.8
5	Testosterone ng/ml	5.0 ± 0.6	3.2 ± 0.4

Hormonal profile indicates decrease ($P > 0.05$) in the hormonal concentration of the test rats on coffee extract feed compared to their corresponding controls.

Table 3: Haematological and haemostatic profile of albino rats before the administration of coffee extracts and 28 days after the administration of coffee extracts

Extracts	Hbg/dl±S.D	PCV%±S.D	WBC permm ³ S.D	Platelets x10 ⁹ /l±S.D	Bleeding time min±S.D	Clotting time min±S.D	Prothrombin Time sec±S.D	Partial Thromboplast in time
Control Rats Group 1 n=12	13.4±1.8	41.0±0.6	5,700±40	220±52	2.4±0.5	5.0±0.3	12.0±0.6	24±1.4
Test rats before extract administration n=24	13.5 ± 0.7	40 ± 1.5	5,480 ± 25	225±30	2.6±0.7	5.2±0.4	11.0 ± 0.8	21 ± 0.8
Test rats 28days after the administration of coffee extracts	9.0 ± 1.2	28 ± 0.5	3,100±13	120 ± 15	12.5±0.2	18±0.6	35±1.2	52±1.6
P value	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

There are indications from the result in this table 3 that Coffee decreases haemoglobin concentrations and other haematological profiles ($p < 0.05$) and prolongs bleeding time and other haemostatic profiles ($p < 0.05$).

Table 4: Effect of Coffee extracts on the Liver function indices and Protein Level of female Wistar rats.

Groups	Total Bilirubin mg/dl ±S.D	Conjugated Bilirubin mg/dl±S.D	ALK.Phos lu/L±S.D	ASP.Trans lu/L±S.D	ALanin Trans iu/L±S.D	Protein mg/dl ± S.D	
						Total	Albumin
Control Group 1 n=12,extract free	0.05 ± 0.00	0.12 ± 0.00	48 ± 0.4	9.2 ± 0.4	8.6 ± 0.5	70 ± 2.5,	36 ± 1.4
Test rats before Coffee extract administration	0.05 ± 0.00	0.10±0.00	50±1.0	12.2±0.6	9.0±0.6	72±1.8	38 ± 1.2
Test rats 28days after Coffee extract administration	0.08±0.00	0.2±0.00	30±1.4	11.0±0.5	9.2±0.3	71±1.5	35 ± 1.2
P value	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

There was significant effect of Coffee extract on alkaline phosphatase causing a decrease while other parameters were not affected.

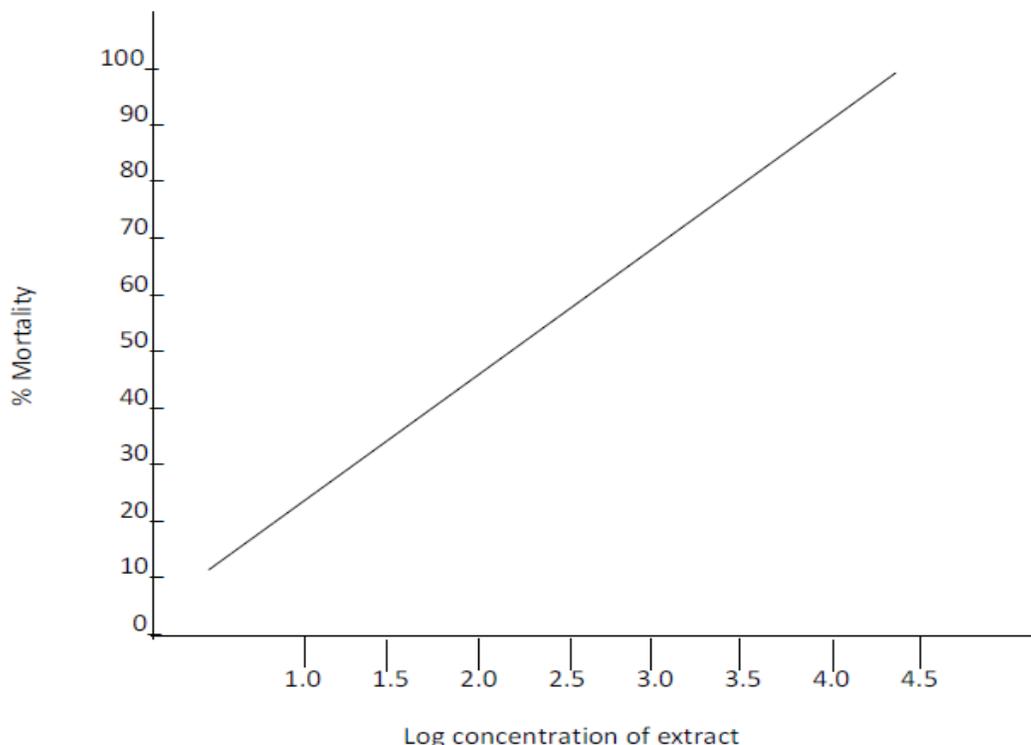


Figure 1: Lethality studies(LD₅₀) of Coffee beans(Arabica)

The LD₅₀ of rats on coffee extract (Arabica) gave 2.00mg/kg.

DISCUSSION

Fertility hormones are those hormones involved in reproduction, formation, and development of the primary and secondary sex organs, regulate the reproductive cycle in women and also play important roles in pregnancy and probably parturition.

The activities of Coffee in the fertility hormones, haemostatic and biochemical profile of female albino Wistar rats have been studied. The phytochemical analysis of the coffee extract showed the presence of alkaloid, caffeine, phenols, calcium, carbohydrates, terpenoids, sugar, fats and oil, tannins while resins acidic compounds, glycosides were absent. Phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against diseases or more accurately, to protect against diseases (Olatunde Ahmed et al 2014).

The caffeine present in coffee stimulates the central nervous system, affecting the brain and spinal cord. Two natural occurring natural coffee compounds, Kahweol and Cafestol, both oil components in coffee are believed to have anti - carcinogenic properties which could be responsible for a reduction in the risk of liver cancer. Also present in coffee are two plant phenols chlorogenic and caffeic acid which have antiviral properties and to be capable of preventing replicable of the Hepatitis B virus. This could also have a potential role in coffee's effect on the liver.

The acute toxicity study of coffee extract showed that the extract was nontoxic as shown by the LD₅₀(Fig.1) of 2,000mg/g. The dose used in this study (5mg/g) was hence lower than the lethal dose and so was the period of study. (Oguwike et al, 2014). Female fertility depends on the hormonal changes in the body. There is an expected level of reproductive hormone particularly the follicle stimulating hormone and luteinizing hormone that should be present to allow ovulation to occur. Any increase or decrease in this normal level will determine the presence of a disease condition.

According to the result obtained in table 2, coffee consumption decreases the level of hormones and when the level of follicle stimulating hormone and luteinizing hormone is below normal, it can lead to infertility in both male and female. Low levels of the hormones may be due to decreased secretion of gonadotropin releasing hormone from the hypothalamus or due to the failure of the anterior pituitary gland to respond to the gonadotropin releasing hormone stimulation. The decrease in these hormones affects the reproductive cycle. The surge of luteinizing hormone leads to the onset of ovulation and development of the follicles. LH and FSH are responsible for ovarian follicular stimulation, they are produced by the anterior pituitary gonadotrophic, and they play a role in

the maturation of gonads, gametogenesis and steroidogenesis. Progesterone is involved in the female menstrual cycle, pregnancy and embryogenesis of humans. Testosterone is the principle androgen in the circulation of mature male mammals; it is capable of developing and maintaining masculine sexual characteristics (including the genital tract, secondary sexual characteristics and fertility). All these hormones work hand in hand, one either regulating the secretion of the other or mediating the synthesis /production of the others. Decrease or deficiency of any of these hormones can lead to irregularities of cycle (female cycles) or poor development of the sexual organs.

Coffee extracts decreased haematological indices and prolonged bleeding time, clotting time, prothrombin time and partial thromboplastin time (Table 3). The reduction in haemoglobin concentration is because coffee causes blockage of iron thus causing effects on the haemopoietic system and slowing the progressive production of RBC resulting to a decrease in blood level and haemoglobin concentration.

The calcium and alkaloids (protein precipitants) contained in this coffee extracts (Table 1) are very minute and therefore could not cause the precipitation of coagulation factors (Taofeeq et al, 2005). Caffeine binds to adenosine receptors to inhibit adenosine and it inhibits the aggregation of platelets. The study also showed that the daily administration of coffee extract to the test rats decreased their alkaline phosphatase but do not have any effect on alanine, transaminase, aspartate, transaminase, protein concentration and bilirubin (Table 4). Thus suggesting that coffee drinking is related to reduced risk of liver diseases, hence suggesting a continuum of favorable effects of coffee on liver function.

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Cite this Article: Oguwike FN, Chukwu CL, Oraegbunam SC, Eze RI (2016). Activities of Coffee in the Fertility Hormones, Haemostatic and Biochemical Profile of Female Albino Wistar Rats. *Greener Journal of Medical Sciences*, 6(3): 046-051, <http://doi.org/10.15580/GJMS.2016.3.052016094>