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Original Research Article

Effect of Caffeine on Stress Biomarkers on Male Wister Rats

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Abstract

Caffeine is widely consumed by the world population because its presence in major constitute of beverages like coffee and tea. This study was aimed at determining the biochemical effect on caffeine consumption in male wistar rats. This was a case-control study of the effect of exposing 180gram-220gram male wistar rats to caffeine for 3 days. Blood cortisol, FBG and FBC were measured in all the animals. Serum cortisol was significantly lower in the control group ($P<0.001$) and there was a significant weight loss in the caffeine-treated rats ($P<0.001$). Caffeine consumption had a significant direct correlation with weight ($r-0.88$ $p<0.001$). Similarly, increase cortisol level was significantly associated with increase in caffeine consumption ($P>0.002$).

Keywords: Caffeine, coffee and tea, male wistar rats, Blood cortisol.

INTRODUCTION

1.1 Background to the Study

Stress can be defined as a mentally or emotionally disruptive or upsetting condition occurring in response to adverse external influences and capable of affecting physical health, usually characterized by increased heart rate, a rise in blood pressure, muscular tension, irritability and depression. (American Heritage (R) Dictionary, 2009).

The NPR/Robert Wood Johnson Foundation/Harvard School of public health burden of stress in American survey was conducted from March 5th to April 8, 2014 with a sample of 2,505 respondents. The survey examines the role stress plays in different aspects of Americans lives including the public's personal experience of stress in the past month the perceived effects of their stress causes of that stress, their method of stress management and their general attitudes about effects of stress in people's lives. Over (43%) reported stressful events or experiences related to health. including 27% who mentioned illness and disease and 16%1 who said the\ had experienced the death of a loved one.

Caffeine is a widely consumed drug without knowing its effects on stress biomarkers. Caffeine is a drug that is maturely produced in the leaves and seeds of many plants. It's also produced artificially and added to certain foods. Caffeine is defined as a drug because it stimulates the nervous system.

Methyixanthine caffeine, present in many common beverages, is among the psychostimulant drugs more extensively consumed all over the world. Besides having a stimulant effect on the heart and respiratory system, caffeine also presents numerous biochemical effects. It has weak reinforcing properties inducing self administration both in humans and non-human primates (Biol Res *et al.*, 2002). Moderate doses of caffeine induce behavioural stimulant effects that suggest CNS stimulation but higher doses can result in change in the biochemical processes in the body. One of the biochemical processes affected by caffeine is cortisol production.

In the last four decades, several attempts had been made to examine the effects of caffeine on human beings. Previous attention had been directed towards its effects on motor responses, visual monitoring (Baker *et al.*, 1972) Nash, 1962) Wakefulness; (Goldstein, *et al.*, 1965) tolerance; (Coltoii *et al.*, 1967). Regina, *et al.*,

(1974; human performance, motor coordination, judgment and mood (Weiss and Lathes (1962). Human cardiac response has been subjectively reported to be affected by caffeine ingestion. A significant effort has also been directed on the effect of coffee on Blood pressure; (Freestone and Ramsay, 1982; Periti, *et al.*, 1987; Lane. *et al.,* 1998; Al' absi *et al.,* 1998). Groliman (1951) pointed out that although ideas become clearer, thought flows more freely and fatigue and drowsiness disappear, caffeine ingestion often leads to inhibition of logical, connected thought, and much effort is required to focus attention on a single object.

Studies using laboratory animals suggests that cafleine afiects cortisol by increasing its production and elevating its levels resulting in stress conditions. These studies support clinical evidence of caffeine- induced stress, tolerance on continued use, and withdrawal anxiety in chronic caffeine-containing beverage users (Bhattachaiya *et al.,* 1997).

Several authors (Winstead, 1976; Nehlig, *et al.,* 1992; RaIl, 1980 and AL' Abs, 1998), have corroborated the ability of caffeine to activate the central nervous system and increase the circulating catecholamines and free fatty acid.

Caffeine has been described as an almost idea stimulant in that it enhance cognition and motor activity and produces few or no side effects for most people (Thompson, 1975). The clinical literature on psychological and physiological reactions to caffeine and caffeine containing beverages is extensive especially in Nigeria, whereas, the literature on it's biochemical effect are spare.

Caffeine is not without its side effects. behavioural effects are partially dependent upon the tolerance level of user, which is increased b\ chronic usage. Doses that increase tolerance result in indigestion; nervousness and insomnia (Leukel, l972 Childs. 1978). Further research has confirmed a significant relationship between excessive caffeine consumption and high level of self-reported anxiety and stress (Winstead, 1976, Greden *et al.,* 1978).

Experimental Materials used:

- Male wistar rat
- Chloroform
- Syringe
- Distilled water
- Surgical gloves
- Weighting balance
- Cotton wool
- Dissecting board
- Beaker
- EDTA bottle
- Glucose oxylate
- Flouride oxylate
- Cortisol reagent
- Glucose reagent
- Dissecting blade

3.2 Animal Preparation and Sample Collection

A total of sixteen (16) healthy male albino wistar rats of weight ranging from 1 8Ograms to 22Ograms were used for this study. These rats were all feed and housed in the preclinical animal house, Faculty of Basic Medical Science, University of Port Harcourt.

The animals were kept and nurtured under laboratory conditions, temperature, humidity, light and were all owned free access to food and water as well. The experimental protocols and procedures used in this study were approved by the ethical committee, University of Port Harcourt rivers state, Nigeria and conform to the guideline of the care and use of' animals in research and teaching.

The animals were divided into two:

- Treatment group and the
- Control group.

The treatment groups were made of twelve (12) animals and they were given 18mg/kg of caffeine and food for 72 hours. While the control groups were made up of four (4) animals and they were given food and water.

The blood samples were collected from the jugular vein with syringe and dropped on EDTA glucose oxylate bottle and fluoride oxylate bottle.

Inclusive Criteria: Healthy adult male wistar rats.

Exclusive Criteria

- Sick animals
- Female animals
- Under weight animals

3.3 Acclimatization of Animals

After identification, the animals were weighed using a zhengya weighing balance and housed in a wire mesh cage with the 12 hours light darkness cycle, for two weeks so as to acclimatize to the environmental condition of the University of Port Harcourt, the study was generally conducted in accordance with recommendation from the 1983 declaration of Helsinki on guiding principals in the care and use of animals.

Experimental Animals in the Cage

3.4 Sacrifice of Animals

Sixteen (16) rats were sacrificed and before sacrifice, the rats were weighed and anaesthetized with chloroform. After sacrifice, some b1ood were taken from the jugular vein.

Sacrifice and Sample Collection

3.5 Procedures and Principles for the Experimental Assays

- Blood Glucose
- Procedure
- The blood glucose level was estimated using glucose oxydase method.

Principles

Glucose oxidase catalyses the oxidation of β-D-glucose δ-lactone with the concurrent release of hydrogen provide, in the presence, of peroxidase (POD) this hydrogen peroxide (H_20_2) enters into a second reaction involving p-hgydroxybenzoic acide and 4-aminoantipyrine with quantitative formation of a quinoneimine dye complex which is measured at 5l0nm.

The reactions involved are:

- 3-D- glucose + O_2+H_2O $^{(GOX)}$ D-glucono-δ-lactone + H_20_2
- $2H_20_2$ + P—droxybenzoicacid + 4-amino-antipyrine $^{(POD)}$ quinoneimine dye +$4H_20$

Result Calculation

Absorbance of test X standard concentration of glucose

Absorbance of standard

- Standard concentration of glucose is 5.6mnol/L

Cortisol Procedure

Cortisol level was estimated using ELISA microwells method.

Principles

Competitive enzyme immunoassay

The essential reagents required for a enzyme immunoassay include: antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated

antibody enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation;

$^{Enz}Ag+Ag+ Ab_{Btn} \rightleftharpoons Ag\ AbBT+AgAb_{Btn}$

Ab_{Btn} = biotinylated antibody (constant quantity)
Ag = native antigen (variable quantity)
^{enz}Ag = enzyme-antigen conjugate (constant quality)
$AgAb_{Btn}$ = antigen-antibody complex
$^{enz}AgAb_{Btn}$ = enzyme-antigen conjugate-antibody complex
ka = rat constant of association
ka = rate constant of dissociation
k=ka/ka = equilibrium constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilize on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{enz}AgAbB_{Btn} + streptavidin_{ew}$
Immobilized complex $streptaidin_{ew}$
- streptadin immobilized on well immobilized complex = sandwich complex bound to the solid surface.

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration by utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Red Blood Count
Procedure

A 20m1 volume of whole blood was added to 3.95m1 of hayem fluid and agitated in a plain bottle neubauer's haemalytomer slide (counting chamber! was prepared and the solution was introduced at 45° and left on a wet surface between 3.5mins.

The counting chamber was placed on the stage of the microscope and view with 40x lens. The red blood cells in the four squares and Central Square of "R" was counted.

Calculation of Result

Total RBC was calculated using the formula below

$$\text{TotaI RBC} = \frac{n \times DF \times D}{A}$$

Differential count (type text)

Principle

The blood specimen is diluted (usually 200 times) with red cell diluting fluid which does not remove the white blood cells but allows the red cells to be counted under magnification in a know volume of fluid.

Finally, the number of cells in undiluted blood is calculated and reported as the number of red cell/pi of whole blood.

White Blood Count

A 0.38µl volume of Turk's fluid was added to 20µ of blood and agitated in a plain bottle. Neubauer's haemocytomer slide (counting chamber) was prepared and the solution was introduced at 450 and left on a wet surface between 3-5mins.

The counting chamber was placed on the stage of the microscope and viewed with 40x lens. The white blood cells in the four "W" corner squares of both chambers were counted.

Calculation of Result

$$\text{Total WBC} = \frac{n \times DF \times D}{A}$$

n = number of WBQ counted
DF = dilution factor (1:20)
A = depth (0.1)
Differential count

Principle

Whole blood is diluted with a 3% acetic solution which hemolyzes mature erythrocytes and facilitates lenkocyte counting. The standard dilution for lenkocyte counts is 1:20. This dilution is prepared using the lenkocyte unopette system. The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on hymacytometer. The cells are allowed to settle and then are counted in specific areas of the hymacytometer chamber under the microscope. The number of lenkocytes is calculated per µL(x10 9/L) of blood.

Haemoglobin

Two-third of the capillary tubes were each filled with blood from the serum references and control specimens, then one end was sealed with a sealant.

The tubes were then place in the micro-haernotocrit centrifuge with the sealed and facing away from the centre of the centrifuge and centrifuged at a speed of 10,000 n-np for 5mins. The reading was taken using a macro-haematocrit reader.

$$\text{Determination of haemoglobin}$$
$$\text{Haernoglobin} = \frac{PCV}{3}$$

Principle

A hemoprotein composed of globin and heme that gives red cells their characteristic color; function

primarily to transport oxygen from the lungs to the body tissue. The red cells are broken down with hydrochloric acid to get the haemoglobin into a solution.

Monocyte

Procedure

30-40m1 of blood from normal healthy human volunteers, giving informed consent, was collected in sodium citrate (3.8% w/v; 1ml/10ml blood) by venipuncture. Blood was mixed 1:1 with PBSA, layered over histopaque-1077 (three parts diluted blood to two parts Histopaque) and centrifuged (500xg) at 25C for 30mm. The mononuclear cells collected from the plasma hypaque interface were washed once in PBSA, and finally resuspended in 4ml of PBSA.

A percoll solution was prepared by mixing l.65m1 of 1.65ml of 10x Hanks balance salt solution with adjusted to 7.0 using approximately 30µl of 0.1µHCL, with gentle string. 8rn1 of percoll solution were added to 4m1 of mononuclear cells in PBSA in a 10 xl.5cm round-bottom polypropylene tube which had be previously silanized with surfasil auuordi. tu manufacturer's instructions. Mononuclear cells were mixed with the percoll solution by inverting the tube 3-4 times then centrifuged at 370xg (1500rpm) in affixed angle SM-24 rotor in a sorvall RC2-B centrifuge at a room temperature for 25mins without braking; alternatively eckmann J2-2 I centrifuge with a fixed angle JA14 rotor can be used at 345xg (I500rrnp) with same results black tubes containing density marker beads, PBSA and the percoll mixture were also centrifuged.

Monocytes appeared as a cloudy layer in the 5mm of the gradient. The monocytes layer was collected, diluted to 50m1 with PBSA and centrifuged at 500xg for 5mms. The cell pellet was resuspended in RPMI —, BSA (2mg/mi) or PBSA and counted.

Cell viability was assessed as the ability to exclude trypan blue. The purity of the monocytes was determined by differential counts of Wright's stained cytocentrifuge preparations (Shandon. Pittsburgh, PA) and by staining for nonspecific esterase using the 2.-napthyl-acetate esterase kit according to manufacturer's instructions.

3.6 Precautions

The following precautions were taken in course of the experiment:

1. It was ensured that all the experimental materials were made available before the start of any experimental procedure.
2. Before weigh of the animals. It was ensured that the weighting scale was properly checked for any form of malfunctioning and quickly fixed.
3. The varied administrative equivalence of the animals was calculated based on their weight, before administration.
4. Syringes were never reused or shared between experimental animals, used syringe were discarded immediately after usage.
5. The presence of air-bubbles in the syringe was checked and quickly removed before administration.

RESULT AND ANALYSIS

From the statistical analyzed result, table 4.1 shows the comparism between the mean of the control group and the means of the caffeine group, using student 't' test.

Table 4.2 shows the relationship between caffeine and the biomarkers using spearman's correlation.

Table 4.3 shows the evaluation of the association between serum cortisol, caffeine and all the other biomarkers.

Table 4.1: Biochemical Responses to Stress induce by Caffeine Consumption

Parameters	Control	80mg/kg caffeine		
	Mens (sem)	Means (sem)	T	p-value
Pev	37.86(1.55)	38.50(4.77)	0.17	0.86
Hb	12.64(0.53)	12.82(1.53)	0.14	0.88
Rbc	4.19(0.18)	4.33(0.52)	0.33	0.74
Wbc	9.36(1.17)	11.57(2.01)	0.99	0.33
Neutophils	20.86(2.35)	26.67(7.48)	0.97	0.34
Lymphocytes	76.29(2.31)	69.33(6.99)	1.22	0.23
Monocytes	2.79(0.45)	4.00(1.03)	1.27	0.21
Eosinophils	0.07(0.07)	0.00(0.00)	0.64	0.52
Fbg	3.25(0.34)	4.47(0.70)	1.77	0.09
Cortisol	0.66(0.05)	3.85(057)	8.66	0.001
Weight 1	174.29(4.77)	200.00(5.16)	3.18	0.005
Weight 2	243.33(9.19)	181.67(4.77)	5.95	0.001

Table 4.2: Correlation of Caffeine with other Biochemical Biomarkers

Parameter	Caffeine
Pcv	0.21
Hb	0.19
Rbc	0.20
Wbc	0.25
Neutrophils	0.09
Lymphocytes	-0.22
Monocytes	0.27
Fosinophils	-0.15
Fbg	0.38
Cortisol	0.80**
Weight 1	0.61**
Weight 2	-0.88**

** Correlation is significant at the 0.01 level
* Correlation is significant at the 0.05 level.

Table 4.3: Multiple Linear Regression between Cortisol and other Biomarkers.

Model	Unstandardized coefficients		Standardized coefficients	T	sig
	B	Std. Error	Beta		
(constant)	1.062	3.934		0.27	0.79
Caffeine	3.072	684	864	4.49	0.002
Pev	405	782	1.862	0.52	0.62
Hb	-751	2.369	-1.139	0.32	0.76
Rbc	-1.301	2.545	-660	0.51	0.62
Wbc	067	065	183	1.03	0.33
Lymphocytes	-010	029	056	0.39	070
Monocytes	055	137	056	0.39	070
Eosinophils	432	1.189	058	0.36	0.73
Fbg	076	238	068	0.32	0.76
Weight 1	-006	017	-077	0.37	0.72

a. Dependent variable: cortisol

DISCUSSION AND CONCLUSION

5.1 Discussion

There was a significant increased on cortisol in the treatment group compared to the controls (t = 8.66 and p = 0.001). it also showed a significant correlation with caffeine and there was an association between cortisol and RBC evident by their markedly increase.

A study conducted by ping *et al.*, (2012) showed that at post-caffeine consumption, cortisol level as still high compared to that of the control. Caffeine injected i.p. to rats markedly increased serum corticosterone (Spindel *et al.*, 2001). Another study evaluated the effects of caffeine in habitual smokers in a repeated measure design. It was discovered that serum cortisol concentration was not increased by caffeine (Gilbert *et at.*, 2000). In this study, cortisol levels increased due to increase in caffeine consumption (Gerri French R.D.M.S. CDI: 2004).

There was no statistically different in blood sugar level compared to the control.

In this study, there was a decrease in blood glucose level in the treatment group when compared to the control (t 17, p 0.09) and there was no significant correlation between caffeine level and blood sugar level.

Blood glucose showed a negative correlation with cortisol and although there was an association between glucose and cortisol, but not statistically significant.

This could be due to both stress and adrenal fatigue. Stress signals the body to raise blood sugar level in order to generate energy to respond to stress but if the body cannot meet its demand for blood glucose due to its fatigue adrenals, hypoglycemia can result.

Urzua *et al.*, (2002) showed that there was a significant decrease in glucose level of caffeine treated rats. While a study conducted by Sachs *et al.*, (1984) showed that chronic caffeine intake induced peripheral insulin resistance resulting in high levels of blood glucose. Another study conducted by Lane *et al.*, (2005) tested the effect of caffeine on fasting glucose and discovered that caffeine did not affect the fasting level of glucose compared with placebo.

In this study, the blood glucose showed a decrease in level. This could be due to the short

duration of administration. Dose administered and/or the blood glucose not being measured in the fasting state.

There was no statistical significant different study, WBC showed an increase in level in the treatment group when compared to the control group (t>0.99, P>0.33) and no correlation between WBC and caffeine. This could be due to the white blood cells diuretic effect of caffeine resulting in haemo-concentration.

Similarly, neutrophils showed an increased in level in the treatment group when compared to the control and there was a correlation between neutrophils and caffeine, although not statistically significant.

Red blood cells showed no significant increase in level between the treatment and control group, there was also no positive correlation between RBC and caffeine. However, there was an association of RBC with cortisol by its increased level due to caffeine consumption.

A study conducted by Adrian *et al.*, (2001) showed an increased in nuetrophils and WBC while another study conducted showed a decrease in neutrophis, (Louise *et al.*, 2006). This study showed in increased in the above hematological parameters, agreeing with the study conducted by Adrian *et al.*, (2001) but disagreed with that conducted by Louiser *et al.*, (2006).

In this study, only RBC showed a statistically significant increase in level. This is due to the diuretic effect of caffeine which can result in heamo-concentration.

There was a statistical different in weight in the treatment compared to the control (t=3.18, p=0.005), and a correlation between weight and caffeine.

A study conducted by American journal of Clinical Nutrition in (2006) shows that caffeine intake were associated with weight loss.

5.2 Conclusion

Caffeine consumption induces significant biochemical stress in animals.

REFERENCES

- Bolumar, F., Olsen, J., Rebagliato, M., Bisanti, L., & European Study Group on Infertility and Subfecundity. (1997). Caffeine intake and delayed conception: a European multicenter study on infertility and subfecundity. *American journal of epidemiology*, *145*(4), 324-334.
- Bruce, B., & Spiller, G. A. (2019). Caffeine, calcium, and bone health. In *Caffeine* (pp. 345-356). CRC Press.
- Bruce, M., Scott, N., Shine, P., & Lader, M. (1991). Caffeine withdrawal: A contrast of withdrawal symptoms in normal subjects who have abstained from caffeine for 24 hours and for 7 days. *Journal of Psychopharmacology*, *5*(2), 129-134.
- Christianson, R., Oechsli, F., & Van Den Berg, B. (1989). Caffeinated beverages and decreased fertility. *The Lancet*, *333*(8634), 378.
- Conlisk, A. J., & Galuska, D. A. (2000). Is caffeine associated with bone mineral density in young adult women?. *Preventive Medicine*, *31*(5), 562-568.
- Fenster, L., Eskenazi, B., Windham, G. C., & Swan, S. H. (1991). Caffeine consumption during pregnancy and spontaneous abortion. *Epidemiology*, *2*(3), 168-174.
- Ford, R. P. K., Schluter, P. J., Mitchell, E. A., Taylor, B. J., Scragg, R., Stewart, A. W., & New Zealand Cot Death Study Group. (1998). Heavy caffeine intake in pregnancy and sudden infant death syndrome. *Archives of Disease in Childhood*, *78*(1), 9-13.
- Gupta, V., & Lipsitz, L. A. (2007). Orthostatic hypotension in the elderly: diagnosis and treatment. *The American journal of medicine*, *120*(10), 841-847.
- Kugelman, A., & Durand, M. (2011). A comprehensive approach to the prevention of bronchopulmonary dysplasia. *Pediatric pulmonology*, *46*(12), 1153-1165.
- Nehlig, A., Daval, J. L., & Debry, G. (1992). Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain research reviews*, *17*(2), 139-170.
- Rubin, G. J., & Smith, A. P. (1999). Caffeine withdrawal and headaches. *Nutritional Neuroscience*, *2*(2), 123-126.
- Schmidt, B. (2005). Methylxanthine therapy for apnea of prematurity: evaluation of treatment benefits and risks at age 5 years in the international Caffeine for Apnea of Prematurity (CAP) trial. *Neonatology*, *88*(3), 208-213.
- Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of psychosomatic research*, *53*(4), 865-871.