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## RESEARCH ARTICLE

### HABITUAL CAFFEINE CONSUMPTION AND ITS INFLUENCE ON MYOCARDIAL FIBERS, BLOOD PRESSURE AND OXIDATIVE STATUS IN PREGNANT ALBINO WISTAR RATS.

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#### Abstract

Caffeine is one of the most ingested drugs in the world. Its effects on the cardiovascular system (CVS) may alter the structure and function of the heart. We hypothesized that habitual caffeine consumption during pregnancy will constantly intensify blood pressure (BP) and damage the morphology of the heart. After 21 days of exposure to caffeine treatments of 1mg/kg, 6mg/kg and 15mg/kg respectively dissolved in vehicle, non-invasive BP, histological analysis and oxidative status were determined using CODA, serum and heart tissues. Data were statistically analysed with ANOVA and Newman-Keuls post hoc test ( $p < 0.05$ ). We observed significant increase in SBP and MABP across the three treatment groups and a considerable improvement in anti-oxidant activity. Histology revealed significant left ventricular (LV) wall thickness and some degree of hypertrophy in the treatment groups compared to vehicle. Our results indicate that habitual consumption of higher doses of caffeine project signs of cardiomegaly.

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#### Introduction:-

Caffeine, which is one of the most psychologically active substance ingested in the world increases alertness, by stimulating the brain (Mercer et al., 2012). It is thus considered a central nervous stimulant that acts by interacting with A<sub>1</sub> and A<sub>2</sub> adenosine receptors as a competitive antagonist. Caffeine binds to adenosine receptors and inhibit the action of adenosine. Adenosine controls the release of norepinephrine, dopamine, acetylcholine, serotonin, glutamate and gamma-aminobutyric acid (GABA). Caffeine therefore inhibits neuronal activity of cholinergic, glutamatergic and GABAergic neurons in the brain, by indirectly affecting the secretions of these neurochemicals (Uddin et al., 2014; Loomans et al., 2012). The binding of caffeine at the adenosine receptors has been reported to cause mild central nervous system (CNS) stimulation (Daly et al., 1999).

Caffeine is mainly found in coffee (*Coffea arabica*), kola nuts (*Cola acuminata*), tea (*Thea sinensis*) and cocoa bean (Committee on military nutrition research, food and nutrition board, 2001). Also, caffeine is present in energy drinks and prescription drugs such as analgesics, appetite suppressants, decongestants and diuretics (Echeverri et al., 2010).

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Studies have suggested that regular consumption of caffeine is associated with adverse cardiovascular (CV) effects (LaCroix et al., 1986; Panagiotakos et al., 2003; Happonen et al., 2004). It has been reported to increase heart rate (HR) and blood pressure (BP) by the stimulation of sympathetic nervous system (Arno et al., 2011). Caffeine has a powerful and continual intense constrictor effect, which could increase the risk(s) of CV disease through its effects on BP. It is known to affect BP through adenosine receptor inhibition and an increased release of selected neurotransmitters such as adrenaline and noradrenaline (Amaresh et al., 2011).

Tofovic et al., (2002) stated that tolerance was not developed to the hemodynamic effects of caffeine in regular caffeine consumers. The significance of this is that for each time caffeine is ingested, it mildly raises BP and HR irrespective of how often it is consumed. In contrary, older studies suggested that habitual use of caffeine leads to the development of tolerance to its physiological effects on BP (Ammon et al., 1983; Robertson et al., 1984).

Studies have reported caffeine to exhibit protective ability against cellular damage (Krisko et al., 2005), thus effective in preventing LDL oxidation (Lee, 2000), reduces MDA levels, augments the activities of hepatic catalase and superoxide dismutase (SOD) (Mukhopadhyay et al., 2003) and possess anti-inflammatory effect (Whayne, 2015; Chen et al., 2010; Lvet et al., 2010). Others suggests that caffeine is able to induce certain forms of oxidative damage by increasing lipopolysaccharide (LP) (Dianzani et al., 1991; Al Moutaery et al., 2003), increased MDA levels in the livers of rats (Karas et al., 2001), reduced Na<sup>+</sup>/K<sup>+</sup>-ATPase activities and increased NO levels by raising the endothelial NOS expression in the kidneys (Umemura et al., 2006; Lee et al., 2002).

Caffeine has been found to increase the aortic stiffness and aortic pressure in healthy and hypertensive adults (Vlachopoulos et al., 2005; Papaioannou et al., 2005; Mahmud et al., 2001).

Although caffeine has been proven to have CNS and gastrointestinal benefits (Schardt, 2008), its effects on the heart has been a source of debate (Pelchovitz and Goldberger, 2011). Some researchers claim that cardiac toxicity can be induced by excess caffeine consumption. On the other hand, it has been observed that caffeine possess antioxidant properties which benefits CV function.

With caffeine consumption linked with altered heart physiology, it is quite possible that CV abnormalities could occur as a result of regular caffeine use in humans. This study is therefore aimed at ascertaining the influence of caffeine on BP, myocardial fibres and oxidative status in pregnant condition.

## **Materials and Method:-**

### **Ethics Statement:-**

All animal handling and experimental protocols implemented in this study was in conformation with the international principles for laboratory animals as obtained in the Helsinki's Declaration (NIH, 1985). Also ethical clearance was obtained from Babcock University Health Research Ethics Committee (BUREC).

### **Animals/Experimental Design:-**

Twenty four (24) pregnant Wister rats with average weight of 115 grams were used in this study. They were obtained and housed in standard plastic cages at room temperature at the animal house, Babcock University, Ilishan, Nigeria. They were exposed to light and dark (12:12) hours and given water and pelletized feed (Caps Feed Ibadan) *ad libitum*.

Virginal smears were taken to determine estrus phases of these animals. Those discovered to be in proestrus phase were exposed to male rats for mating. The day a vaginal plug was discovered or sperm cells found to be present in smear was designated as pregnancy day zero. Pregnant rats were randomized into four groups and treated via oral gavage for at least four days in a week till end of term. Group A provided with vehicle (water), group B was treated with 1mg/kg of caffeine dissolved in vehicle, group C was treated with 6mg/kg of caffeine dissolved in vehicle and group D was treated with 15mg/kg of caffeine dissolved in vehicle. These caffeine treatments mark a circulating blood levels equal to the consumption of between 1-4 cups of coffee in humans (Fredholm, 1995). Caffeine was obtained from Sigma-Aldrich, St. Louis, MO, USA.

BP, cardiac histology and oxidative status were determined on pregnant rats at term. BP was determined immediately after term. 2 heart tissues per group were used for histology and serum was analyzed for oxidative status.

**Blood Pressure (BP) Determination:-**

BP was determined by using non-invasive CODA tail cuff system and a volume pressure recording sensor (Kent Scientific Cooperation, USA). The animal was placed in the rear of the holder and the rear hatch to the holder was secured. The nose cone was in a position to limit the animal from turning around while inside the holder. The animal was allowed about 5 minutes to acclimate to the holder, likewise thermoregulate. Cuff placement, attachment of the cuff to the CODA controller and determination of blood pressure was carried out as described by Daugherty et al., (2009). The systolic and diastolic BP were obtained from the CODA spreadsheet after the cycle was completed. Mean Arterial BP (MABP) was determined by using the formula:  $(SBP + 2 \cdot DBP)/3$ .

**Cardiac Histology:-**

Hearts of experimental animals were fixed by perfusion of hearts with 4% paraformaldehyde solution (obtained from Anatomy Department, Babcock University) containing 150 mM KCl and 5 mM EDTA. Hearts were embedded in paraffin, sectioned, mounted on slides and analysed as described by Wendler et al., (2009).

**Antioxidant study:-**

Lipid peroxidation was measured by the formation of thiobarbituric acid reactive substances (TBARS) using the methods postulated by Beuge and Aust, (1978). The absorbance was measured at 535 nm.

The level of superoxide dismutase (SOD) activity in plasma Liver and heart tissue homogenates were determined by the method of Misra and Fridovich (1972). The catalase activity (CAT) was measured according to the method of Sinha et al., (1972). The exponential disappearance of  $H_2O_2$  was at 570 nm.

Reduced glutathione (GSH) level was determined by the method of Ellman modified by Jollow et al. (1974). The absorbance was measured immediately at 412 nm. The GSH contents were calculated using GSH as standard and expressed as  $\mu\text{mol/mg}$  protein.

**Statistical analysis:-**

Results are presented as means  $\pm$  SEM and comparison of the means was done using the one way analysis of variance, followed by Student's Newman-Keuls post hoc test, using the GraphPad software. A  $p$ -value  $< 0.05$  was considered statistically significant.

**Results:-****Antioxidant Analysis:-**

Malondialdehyde (MDA) analysis in serum and liver tissue revealed that results were significantly lower ( $p < 0.05$ ) in treatment groups compared to vehicle. From the heart tissue MDA analysis, only the group administered 15 mg/kg of caffeine was significantly lower ( $p < 0.05$ ) at  $3.53 \pm 0.22$  nmol/ml compared to vehicle ( $4.78 \pm 0.09$  nmol/ml) and the group administered 1 mg/kg of caffeine ( $4.55 \pm 0.11$  nmol/ml).

SOD and CAT results were significantly higher ( $p < 0.05$ ) across treatment groups for serum, liver and heart tissues when compared to Vehicle.

GSH results for liver and heart tissues were significantly higher ( $p < 0.05$ ) across treatment groups compared to control group. While values for serum (GSH) were  $3.94 \pm 0.14$   $\mu\text{Mol/ml}$  for group treated with 1 mg/kg of caffeine (not significant compared to vehicle -  $3.53 \pm 0.07$   $\mu\text{Mol/ml}$ ),  $5.60 \pm 0.39$   $\mu\text{Mol/ml}$  for group treated with 6 mg/kg of caffeine (significant higher compared to vehicle), and at  $6.62 \pm 0.55$   $\mu\text{Mol/ml}$  for group treated with 15 mg/kg of caffeine (significant higher compared to vehicle).

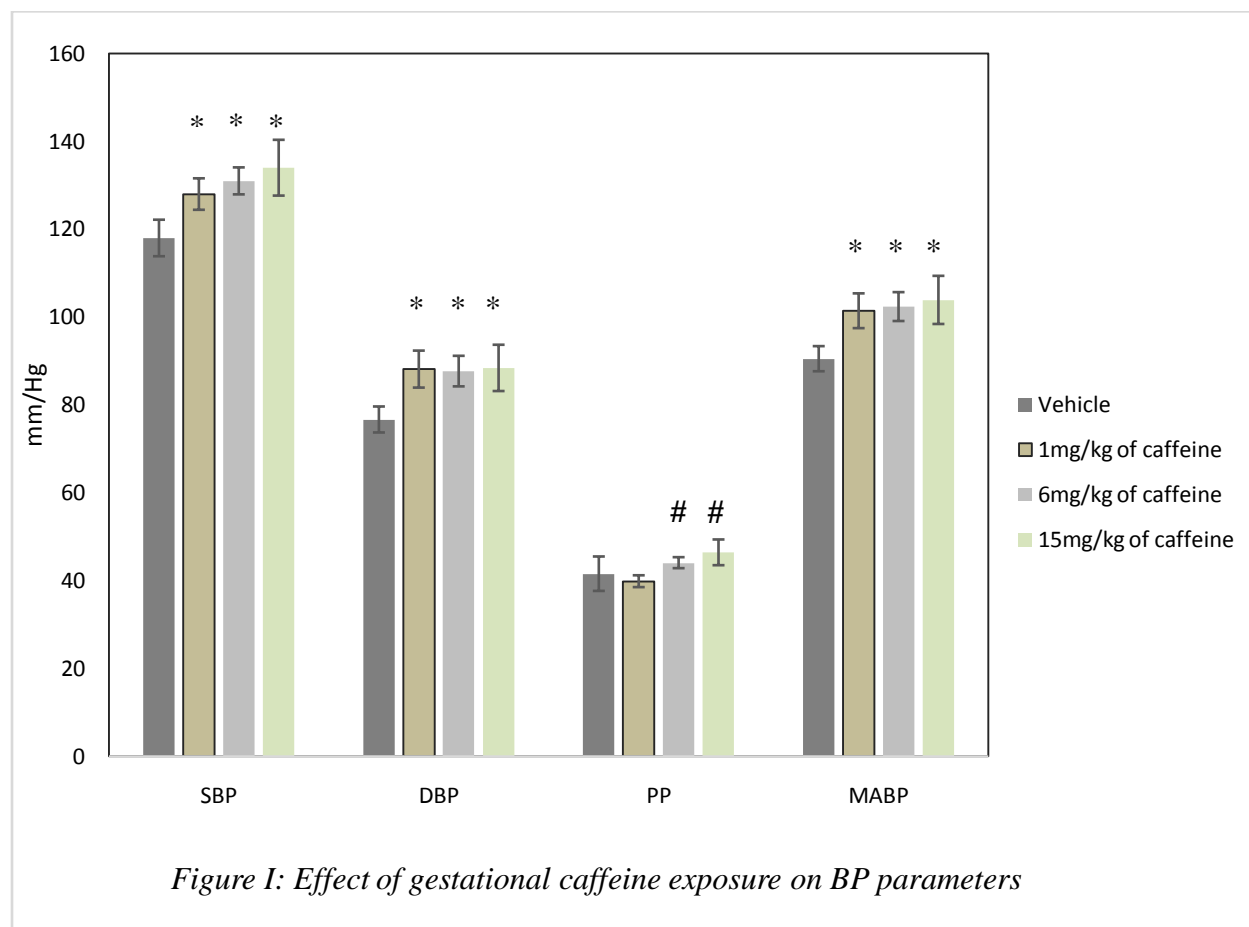
**Blood Pressure (BP) Parameters:-**

Figure I shows values of the systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial blood pressure (MABP), heart rate (HR) and rate pressure product (RPP) for vehicle and the treatment groups immediately after treatment period. MABP, SBP and DBP values were significantly higher ( $p < 0.05$ ) across the three treatment groups when compared to vehicle.

**Histological results:-**

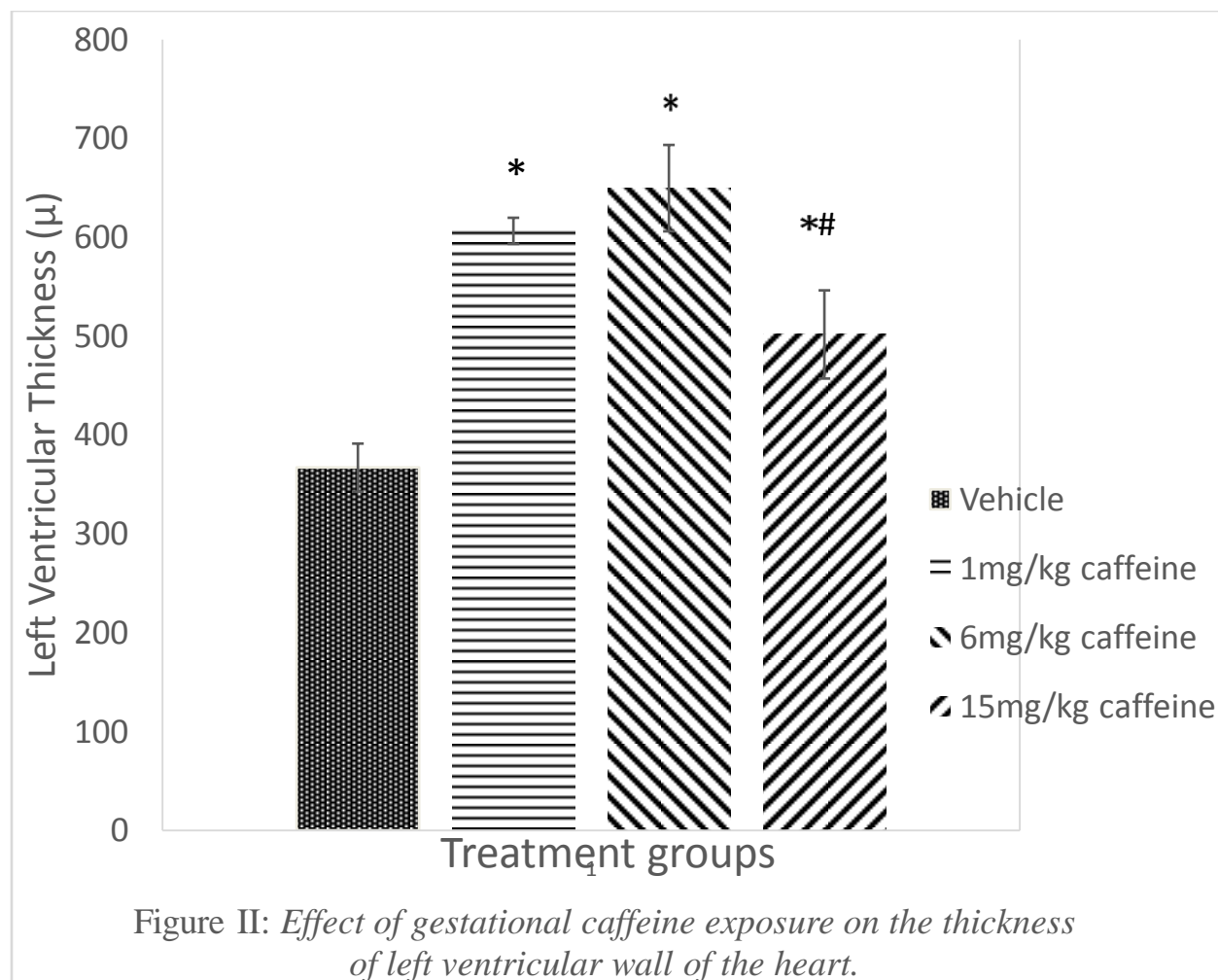
As observed in plate I, Collagen fibers appeared gold orange in colour. Micrograph showed hypertrophic tissue (arrow) a day after delivery even though tissue had good histoarchitecture and fair nuclei distribution (panel B). In

panels C and D, signs of dystrophy (arrows) were observed and panel D showed some level of histoarchitectural disruption. In plate II, fibres appeared to be more in panels B, C and D compared to panel A. Some degree of hypertrophy was observed in panels C and D (arrows). Results for left ventricular wall thickness across treatment groups revealed that thickness was significantly increased compared to vehicle.

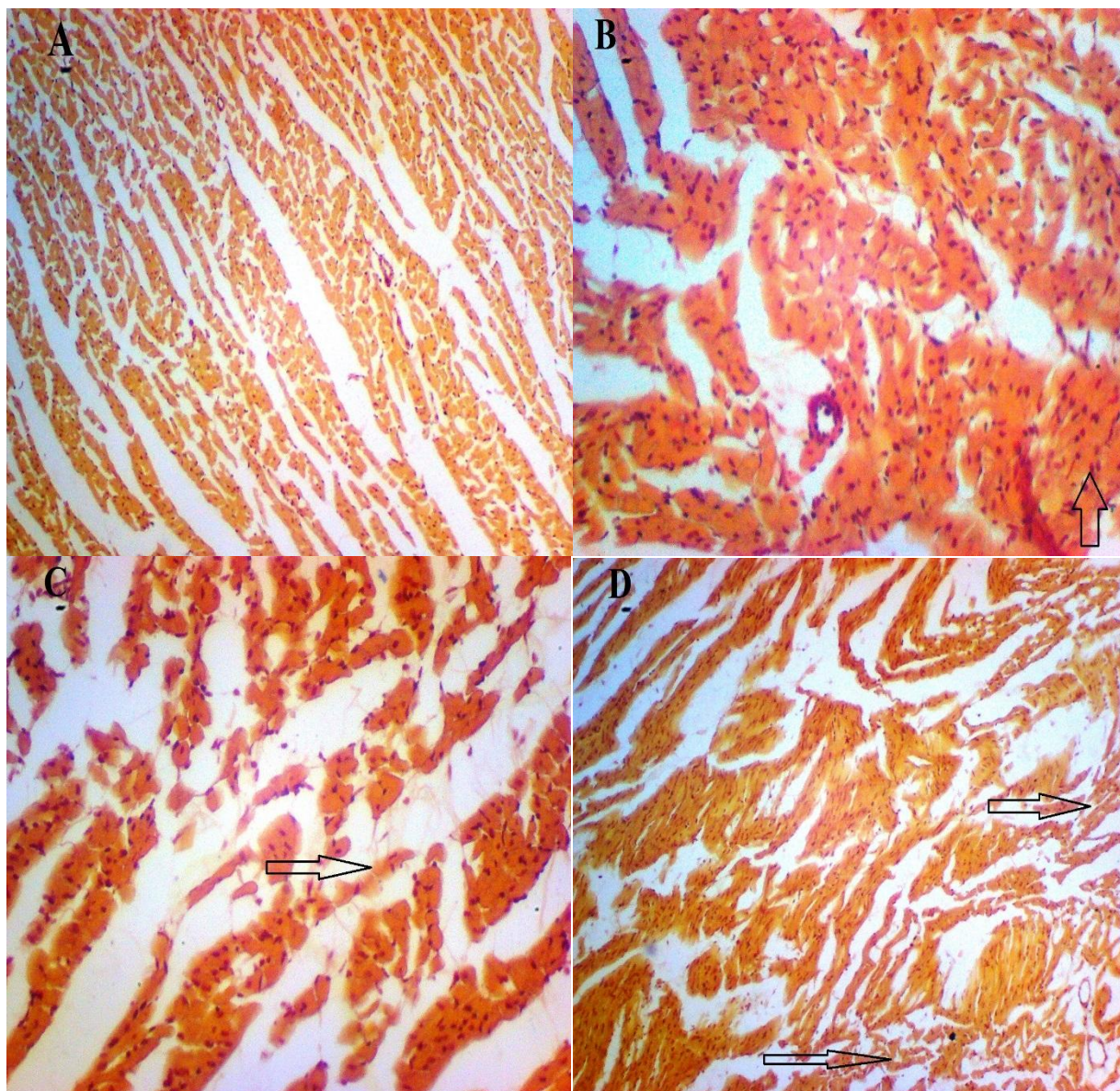


\*= $P < 0.05$  when comparing with the control group.

#= $P < 0.05$  when comparing with the low dose group.







**Plate I:-**Shows histological Weigert's stain of myocardial collagen fibres in the left ventricle (LV) of vehicle (panel A), 1mg/kg of caffeine treated group (panel B), 6mg/kg of caffeine treated group (panel C) and 15mg/kg of caffeine treated group (panel D)x400M. Arrows indicate hypertrophic tissue (panel B) and dystrophy in panels C and D.





**Plate II:-**Shows histological Gordon and Sweet's stain of myocardialreticulin fibres in the left ventricle (LV) of control group (panel A), 1mg/kg of caffeine treated group (panel B), 6mg/kg of caffeine treated group (panel C) and 15mg/kg of caffeine treated group (panel D)x400M. Arrows indicate hypertrophic tissue. Rf – reticulin fibres.

**Table I:-** Oxidative status analysis in serum following modified dose of caffeine treatment.

	Vehicle	1mg/kg of caffeine	6mg/kg of caffeine	15mg/kg of caffeine
MDA (nMol/ml)	10.58±1.32	8.45±0.23*	8.29±0.29*	5.41±0.60*#α
SOD (mg/ml)	4.16±0.17	5.60±0.12*	6.99±0.44*#	8.76±0.81*#α
CAT (mg/ml)	2.11±0.49	3.25±0.12*	4.23±0.52*#	13.27±2.32*#α
GSH (μMol/mg)	3.53±0.07	3.94±0.14	5.60±0.39*#	6.62±0.55*#α

\*= $P < 0.05$  when compared with vehicle

#= $P < 0.05$  when compared with 1mg/kg of caffeine group

α= $P < 0.05$  when compared with 6mg/kg of caffeine group

**Table II:-** Oxidative status analysis in Liver tissue following modified doses of caffeine treatment.

	Vehicle	1mg/kg of caffeine	6mg/kg of caffeine	15mg/kg of caffeine
<b>MDA (nMol/ml)</b>	10.20±0.07	9.72±0.16*	8.55±0.78*#	6.62±0.49*#α
<b>SOD (mg/ml)</b>	4.73±0.13	5.24±0.14*	5.50±0.15*#	5.78±0.21*#
<b>CAT (mg/ml)</b>	2.03±0.66	4.31±0.57*	6.23±0.51*#	8.20±1.13*#α
<b>GSH (μMol/mg)</b>	1.52±0.15	6.98±3.60*	4.88±0.34*	6.65±0.98*α

\*= $P < 0.05$  when compared with vehicle

#= $P < 0.05$  when compared with 1mg/kg of caffeine group

α= $P < 0.05$  when compared with 6mg/kg of caffeine group

**Table III:-** Oxidative status analysis in heart tissue following modified dose of caffeine treatment.

	Vehicle	1mg/kg of caffeine	6mg/kg of caffeine	15mg/kg of caffeine
<b>MDA (nMol/ml)</b>	4.78±0.09	4.55±0.11	4.23±0.53	3.53±0.22*#
<b>SOD (mg/ml)</b>	4.86±0.10	5.65±0.11*	5.88±0.12*	5.99±0.11*#
<b>CAT (mg/ml)</b>	0.27±0.66	14.20±0.57*	17.87±0.51*#	23.80±1.13*#α
<b>GSH (μMol/mg)</b>	0.58±0.15	1.57±0.23*	2.32±0.34*#	4.63±0.98*#α

\*= $P < 0.05$  when compared with vehicle

#= $P < 0.05$  when compared with 1mg/kg of caffeine group

α= $P < 0.05$  when compared with 6mg/kg of caffeine group

## Discussion:-

Caffeine is known to affect several systems of the body. Its use in food and food additives has made it necessary to thoroughly understand its effects on the body system. This study was designed to provide understanding on the effect of sub-chronic caffeine consumption in pregnancy and its effect on some cardiovascular parameters, as well as anti-oxidant status.

After about four weeks of habitual caffeine consumption, BP was significantly raised in the three treatment groups. Increase in BP detected in this study is an indication of no tolerance to caffeine developed after sub-chronic consumption. This is in line with the finding of Tofovic et al., (2002), who reported no tolerance to the hemodynamic effects of caffeine in regular consumers. The rate of metabolism and clearance of caffeine by the hepatic cytochrome P-450 (CYP) isoenzyme is one major factor that can hinder tolerance to caffeine use.

Increase in BP and HR have been described to occur as a result of stress. Stress (likely resulting from caffeine intake), mediates high levels of cortisol, adrenaline and noradrenaline (Echeverri et al., 2010). A continual state of increased stress can chronically raise BP, disturb HR and rhythmicity (Rafetto et al., 2004). Increased BP which was evident in the present study could also be as a result of the inhibition of parasympathetic activity by the antagonism of adenosine receptors (Loomans et al., 2012). Increase in BP was correlated to the doses administered. This may well imply that higher concentrations of caffeine may promote incessant and lingering stress condition and/or unswerving inhibition of parasympathetic activity by the antagonism of adenosine receptors thereby endorsing hypertension, which is one of the major causes of heart failure.

Myosin heavy chain synthesis can increase by 35% within hours after a pressure overload occurrence in heart tissues, leading to an increase in the number of sarcomeres in the myocyte and consequently increase in cardiac muscle mass and thickness (Lorell and Carabello, 2000). Our study reveals significant thickness in the LV of rat heart LV wall, substantial accumulation of collagen and reticulin fibres, as well as increase in the number of reticulin fibres across treatment groups. This is a sign of hypertrophy and increase in cardiomyocyte size. Buscariollo et al., (2014) accounted that the increased wall thickness was consistent with cardiac concentric hypertrophy, characterized by reduced chamber volume.

High BP has been linked with about a 10-fold increase in the incidence of LV hypertrophy (Levy, 1988). This study demonstrates a connection between high BP, increase in cardiomyocyte size, increased LV hypertrophy and wall thickness. These are indications of cardiomegaly (Shi et al., 2014). Reports have indicated that steady rise in BP can stimulate increased collagen synthesis, in same manner consequential fibrosis and hypertrophy (Gordon et al., 1986; Morgan et al., 1985).



The increased BP in the present study cannot be correlated to MDA, SOD, GSH nor CAT. The progressive reduction (MDA) and increase (SOD, GSH and CAT) across treatment groups seem more like the influence of caffeine on these antioxidant enzymes is in a dose dependent fashion. Previous studies have specified that caffeine impedes lipid peroxidation especially in the liver tissues of rats (Kamat et al., 2000; Pasaoglu et al., 2011), instigates and augments the activities of CAT and SOD (Mukhopadhyay et al., 2003).

Caffeine may contain antioxidant properties by instigating oxidative enzymes in the prevention of tissue oxidation and damage. It is also evident that it is capable of giving rise to series of physiological and anatomical mechanisms detrimental to cardiovascular health during pregnancy. Studies will be necessary to ascertain if these effects will be manifested in the offspring.

### Acknowledgement:-

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