LIPID PROFILE AND ANTIOXIDANT ASSESSMENT OF CAFFEINE INDUCED ALBINO RATS TREATED WITH NIFEDIPINE

Fatai A. Kareem*, Ademola O. Akinyemi, Anuoluwapo O. Ajayi & Naomi A. Folorunsho

Department of Science Laboratory Technology, School of Science and Technology,
Gateway Polytechnic, Saapade, Ogun State, Nigeria.
*Corresponding author: akakafy2k@gmail.com; +2348035268959

Abstract

Hypertension is a chronic elevation of blood pressure that, in the long-term results in increased morbidity and mortality. The present study investigates the effectiveness of 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (nifedipine) on lipid profile and antioxidant level of caffeine induced hypertensive albino rats. Twenty-one adult albino rats weighing between 100 and 200g were grouped into three consisting of seven animals each. The control group were fed and orally administered with normal saline, the model and treatment groups were fed and given body weight 100 mg/Kg of caffeine orally for three days while the treatment group was administered with 2ml/kg body weight of nifedipine orally for three days. The blood pressure and body weight were measured accordingly. The animals were sacrificed twenty-four (24) hours after the last administration. The blood sample was collected for assay. There was increase in body weight and blood pressure of the model group when compared with the control while it was restored in the treatment group. The results revealed an increase in total cholesterol and low density lipoprotein level in the model group and restoration in the treatment group. The atherogenic index of the plasma, Castelli's risk index I and II and atherogenic coefficient were increased in the model group while it was restored in the treatment group. There was reduced antioxidants level in the model group and the antioxidants were restored in the treatment group. This study confirmed the antihypertensive potential of nifedipine and therefore can be used as a strong therapy for hypertension with minimum side effects.

Keywords: Antioxidant, blood pressure, calcium-channel blocker, hypertension and lipid profile.

INTRODUCTION

Hypertension is a major cause of morbidity and mortality because of its association with coronary heart disease, cerebrovascular disease and renal disease. The extent of target organ involvement (i.e. heart, brain and kidneys) determines outcome. Studies have shown that hypertension is a major contributor to 500 000 strokes (250 000 deaths) and 1000 000 myocardial infarctions (500 000 deaths) per annum (Rahman *et al*., 2021).

In patients with high blood pressure, the cumulative incidence of first cardiovascular events over 10years is 10% in males and 4.4% in females. Even high normal blood pressure is correlated with an increased risk of death attributable to coronary or cerebrovascular events. Whether treatment of high-normal blood pressure would prevent cardiovascular events is unknown (Haque *et al*., 2019).

Hypertension is recognized globally as a major risk factor for cardiovascular diseases, stroke, diabetes, and renal diseases (Saha *et al*., 2006). About 80% of hypertensive persons have comorbidities such as obesity, glucose intolerance, and abnormalities in lipid metabolism, among others. A prospective study in the northern region of Bangladesh comparing lipid profile status in hypertensive patients as compared to healthy normotensive controls found high serum Total cholesterol, Tryglceraldehyde, and Low density lipoprotein, similar to the study of Islam and Majumder, (2012). Kamrun*et al*. (2014) in their study, observed increased levels of Total cholesterol in hypertensive patients are similar to the findings of some other studies (Anjum*et al*., 2013). However, few studies measured the strong association of hypertension and dyslipidemia among the Bangladeshi population.

Systolic blood pressure and diastolic blood pressure correlated inversely with plasma antioxidant potential (e.g., FRAP) and directly with some oxidative stress-related parameters (e.g., F2-isoprostanes) (Rodrigo *et al*., 2007). Long-term hypertension produces irreversible endothelial dysfunction due in part to self-sustaining ROS production (Touyz, 2004). In patients with already established endothelial damage, measurement of oxidative stress-related

biomarkers will be of less use. In order to establish a functional interpretation of hypertension through oxidative stress-related biomarkers, the early phases of the disease should be studied. In the endothelial dysfunction phase, previous to the development of hypertension, the ROS fluctuations will appropriately reflect the balance between ROS production and their scavenging or depuration by the antioxidant defense system in the endothelium (Ramon *et al*., 2013).

Caffeine is likely safe for most healthy adults when used in doses up to 400 mg daily. This is equal to about 4 cups of coffee. Caffeine is possibly unsafe when used for a long time or in doses over 400 mg daily. Caffeine can cause insomnia, nervousness, restlessness, nausea, increased heart rate, and other side effects. Larger doses might cause headache, anxiety, and chest pain. Caffeine is likely unsafe when used in very high doses. It can cause irregular heartbeat and even death. Products with very concentrated or pure caffeine have a high risk of being used in doses that are too high (Shimizu *et al*., 2016).

Calcium channel blockers can be divided into dihydropyridines (e.g. nifedipine, nimodipine, amlodipine) and non-dihydropyridines (verapamil, diltiazem). Both groups decrease peripheral vascular resistance but verapamil and diltiazem have negative inotropic and chronotropic effects. Nifedipine is effective in severe hypertension and can be used sublingually; there is need for caution because of the risk of excessive hypotension. Calcium channel blockers are often associated with b-blockers, diuretics and/or ACE inhibitors (Trefor *et al*., 2001).

There is conflicting evidence on the effect of caffeine on systemic hemodynamics. In short-term studies, it has been shown to increase blood pressure and peripheral vascular resistance, but tolerance rapidly develops. The pressor effect of caffeine is predominantly due to its action on the resistance vessels rather than an increase in cardiac output. Although the increases in sympathetic nervous system activity, serum adrenaline, and renin have been causally linked, the acute pressor effect is also seen in adrenalectomized patients. Vasoactive hormones such as aldosterone, vasopressin, and arterial natriuretic peptide, however, are not increased by caffeine. Caffeine is a nonselective antagonist of adenosine at both A_1 and A_2 receptors. Adenosine A_1 receptor stimulation inhibits the release of norepinephrine at the sympathetic smooth muscle junction, whereas A_2 receptors have a direct vasodilatory effect (Azra and John, 2001).

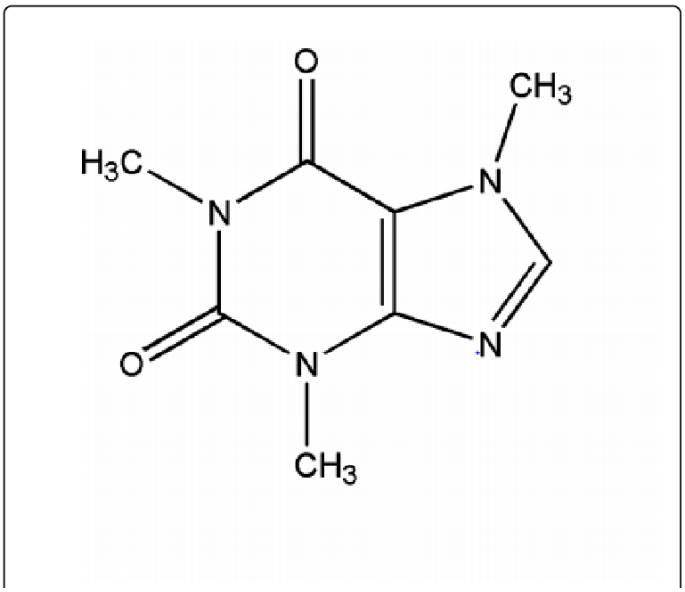


Figure 1: Chemical structure of caffeine (Wachamo, 2017).

The cardiovascular effects of caffeine are mediated largely through blockade of both A_1 and A_2 adenosine receptors. Inhibition of phosphodiesterases is also seen, but only in pharmacological doses. That caffeine may have vascular effects is also suggested in a recent study in which it attenuated the increase in forearm blood flow after exercise. In that study, caffeine also induced an increase in angiotensin II. The main novel finding in our study is that caffeine acutely stiffens the aorta and impedes the function of the peripheral muscular arteries, which may be an additional vascular mechanism for the hypertensive effect of caffeine. On the other hand, there may be some tolerance to this effect of caffeine, an issue that requires a longitudinal study (Azra and John, 2001).

There are evidences that hypertension has direct and indirect relationship with many physiological components of human system; liver function, kidney function, lipid profile, haematological indices and antioxidant enzymes. Therefore, this study evaluates the effect of hypertension on the above listed parameters and the effect of the administration of calcium blocker class of antihypertensive drug (Nifedipine).

MATERIALS AND METHODS

Drugs, Animal and Diet

Caffeine anhydrous was purchased from BDH Ltd, Poole England. Nifedipine tablet used for this project was obtained from Pfizer Global Pharmaceuticals Ltd, Lagos, Nigeria. Twenty one adult wistar strain rats of both sexes, weighing between 100 and 200 g were obtained from the Animal House of the Science Laboratory Department, Gateway (ICT) Polytechnic, Saapade, Remo, Ogun State. The rats were fed with standard rat pellets (Top Feed Nigeria Ltd., Ibadan, Nigeria) and water ad libitum They were housed in individual wire cages in a temperature and humidity controlled room, having a 12-h light and dark cycle.

Experimental Design

The experimental animals were grouped as follow:

Group 1 (Control): seven adult rats were fed with rat pellets and Normal saline throughout the study

Group 2 (model): Seven albino rats were fed with rat pellets and distilled water. They were orally induced with 100mg/kg per body weight of caffeine for three (3) days but were not treated.

Group 3 (treatment): Seven adult rats were fed with rat pellets and distilled water. They were induced with 100 mg/kg per body weight of caffeine for three (3) days and were orally administered with 2ml/100kg body weight of Nifedipine drug for three (3) days.

Procedure for Sacrificing the Animals and Collection of Sample

The blood pressure and body weight of were properly measured before induction, after caffeine induction and after treatment with Nifedipine via animal neonate 1 blood pressure cuff connected with OMRON digital blood pressure monitor and Electronic weighing scale respectively. Twenty-four (24) hours after the last administration, the animals were anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture and then centrifuged while the plasma was kept clean in a specimen bottle stored frozen. The animals were dissected to surgically remove the liver and kidney rinsed in iced cold 0.9% normal saline, blotted dry, homogenized, and later centrifuged. The supernatants were kept in a clean bottle and stored frozen for assay

Measurement of Blood Pressure

The blood pressure was properly measured before induction, after caffeine induction and after treatment with Nifedipine via animal neonate one blood pressure cuff connected with OMRON digital blood pressure monitor.

Measurement of Body Weight

The body weight was properly measured before induction, after caffeine induction and after treatment with Nifedipine via electronic weighing scale respectively.

Biochemical Test

Lipid Profile

Total cholesterol (TC), glyceraldehyde (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) was done according to method described by Kafi, 2014

Brain Antioxidant Assay

Catalase (CAT), Superoxide dismutase (SOD) test, Lipid peroxidation product (MDA) test and Glutathione (GSH) test was carried out according to the method described by Fajobi *et al.*, 2020).

STATISTICAL ANALYSIS

The experimental results were expressed as mean ± standard error of mean (SEM) and the mean were subjected to one-way analysis of variance ANOVA followed by Duncan Multiple Range Test (DMRT). Significant level was set at $p \leq 0.05$ using Graphpad Prism 6.0.

RESULTS:

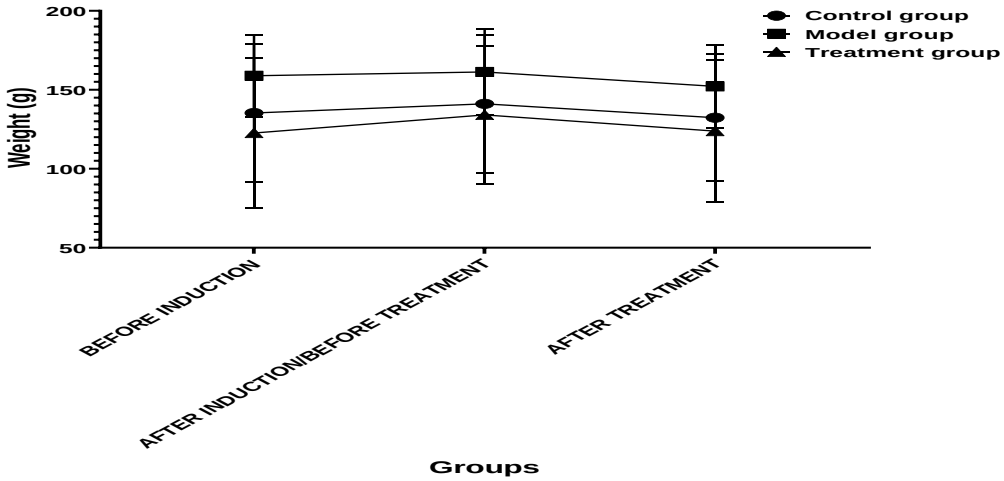


Figure 1: Body weight of experimental animals

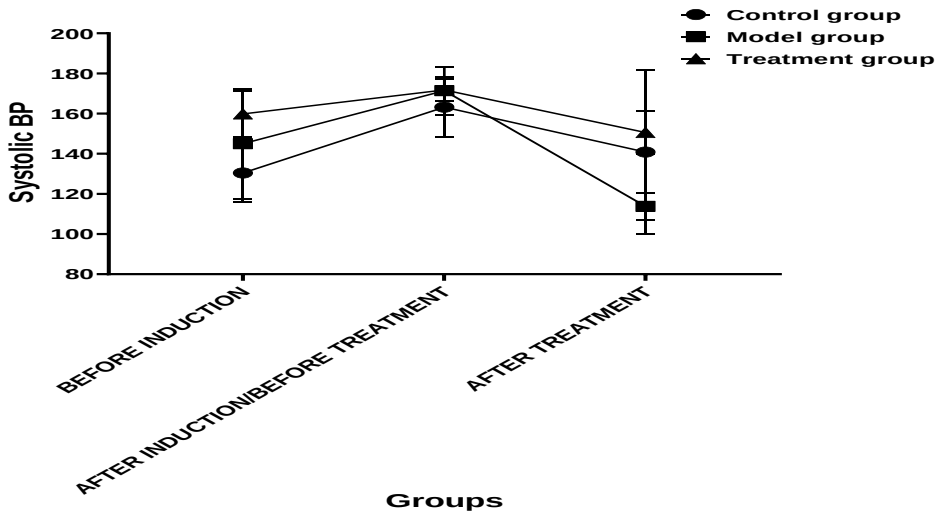


Figure 2a

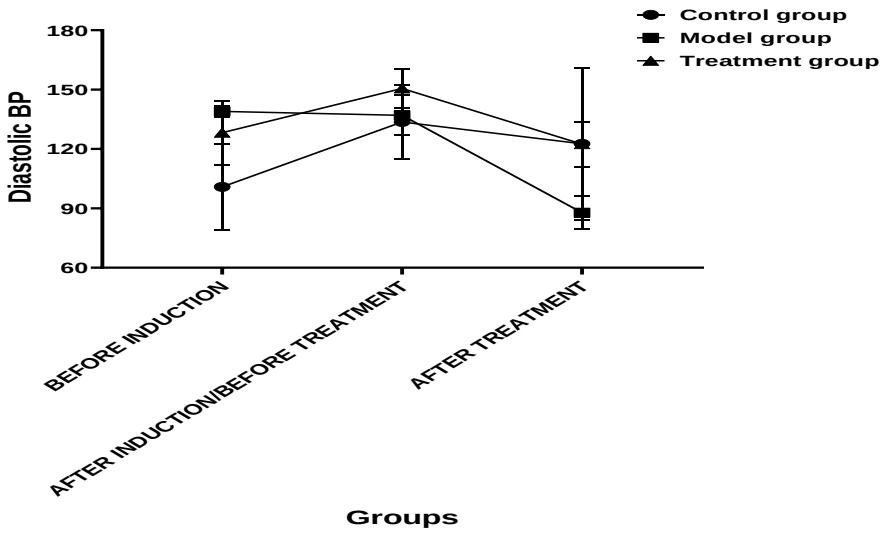


Figure 2b

Figure 2a-2b: shows the systolic and diastolic blood pressure of experimental animal before induction, after caffeine intake and after treatment with nifedipine drug.

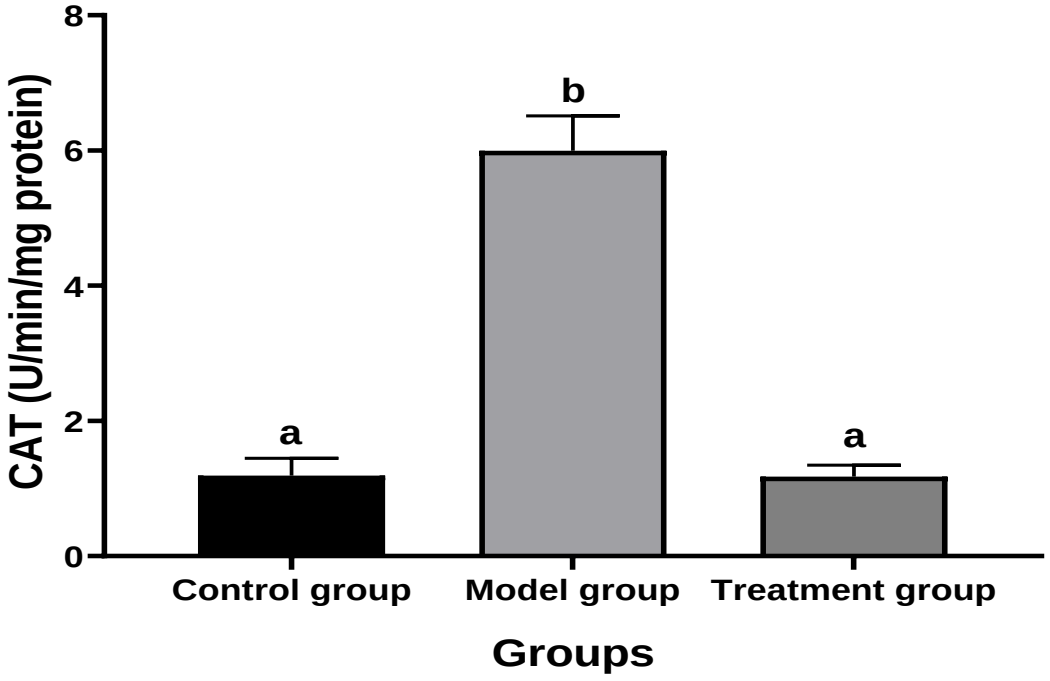


Figure 3a

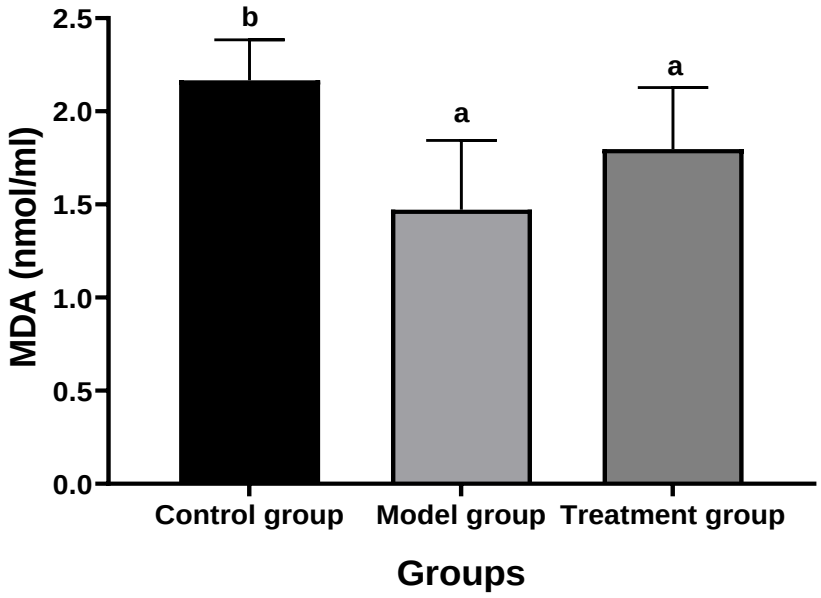


Figure 3b

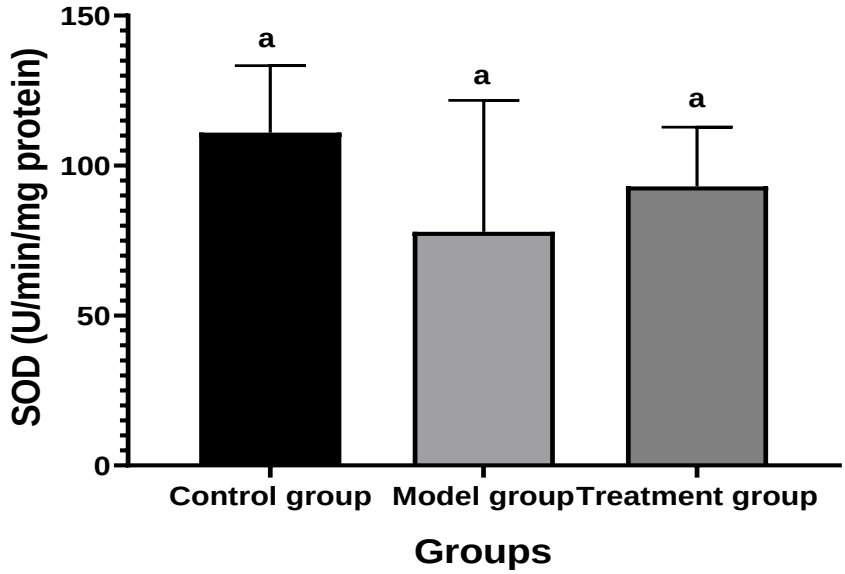


Figure 3c

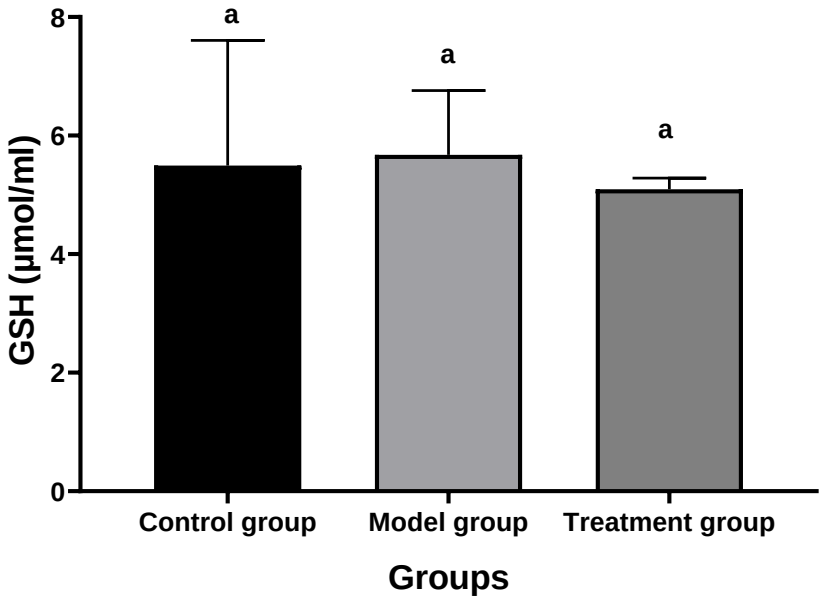


Figure 3d

Figure 3a-3d: Shows the activities of brain antioxidant markers among experimental group

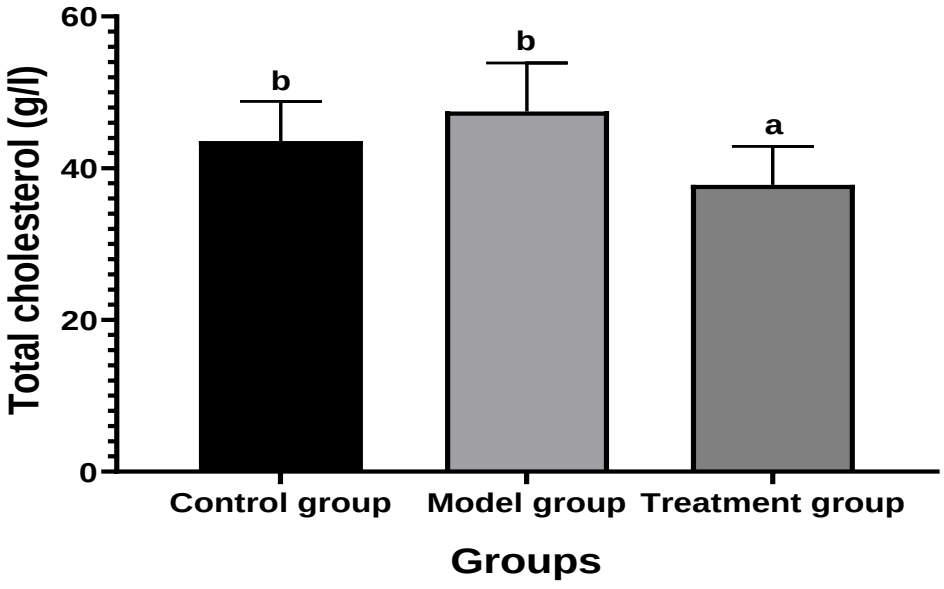


Figure 4a

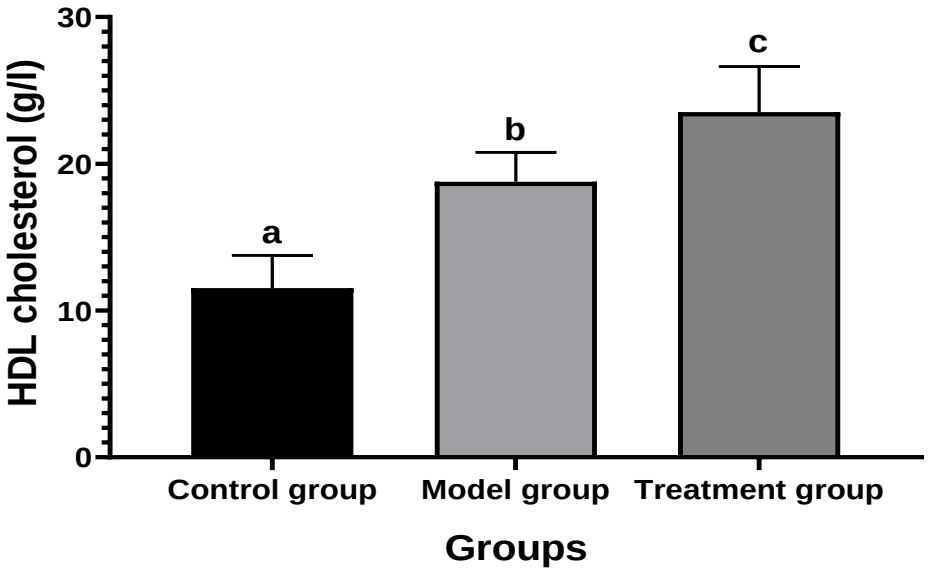


Figure 4b

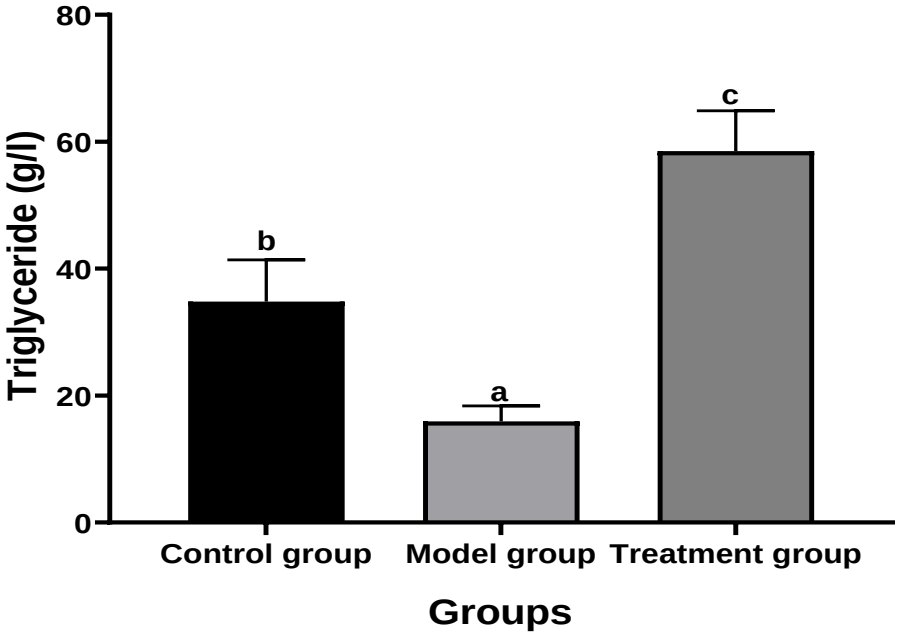


Figure 4c

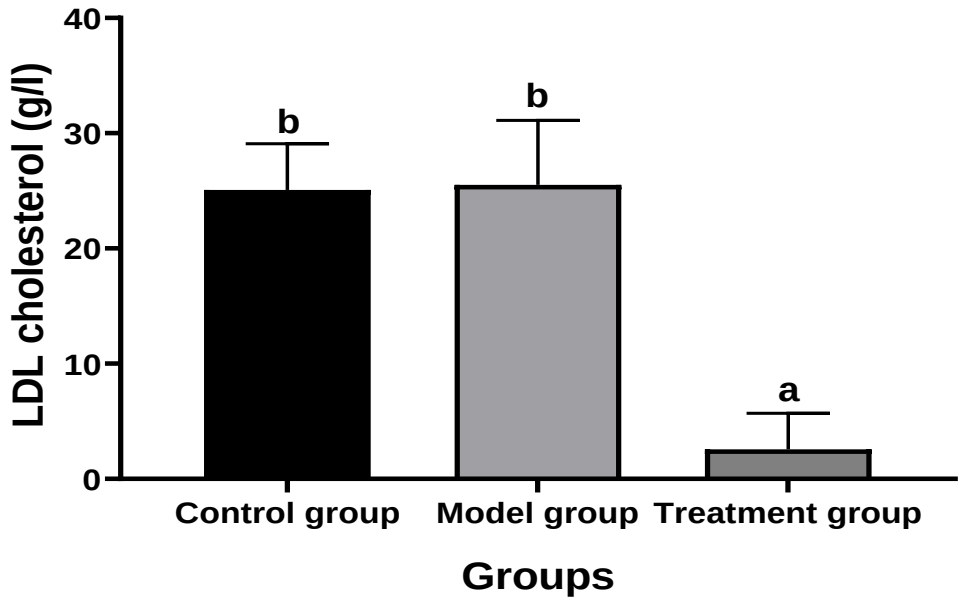


Figure 4d

Figure 4a-4d: showing the analysis of lipid profile among experimental group

DISCUSSION

Hypertension is known to be associated with alterations in lipid metabolism which gives rise to abnormalities in serum lipid and lipoprotein levels. This study investigated the relationship between lipid profile, antioxidant marker enzymes and hypertension.

Caffeine intake simultaneously increased the systolic and diastolic blood pressure while treatment with nifedipine was able to reduce the risen blood pressure. Caffeine intake increased the level of total cholesterol, high density lipoprotein and low density lipoprotein while it caused a reduction in the level of triglyceraldehyde. Administration of nifedipine drug increased the level of trygleceraldehyde, HDL and LDL but lower the level of total cholesterol .

The effect of caffeine intake caused the body weight of the experimental animal to increase after induction when compared with the body weight taken before induction. While treatment with Nifedipine brought about a reduction in the increased body weight caused by caffeine intake.

High dose of caffeine increased the activity of catalase (CAT) and glutathione (GSH) but reduced the activity of malondialdehyde (MDA) and superoxide dismutase (SOD) when compared with the control group. Treatment with nifedipine reduced the activity of catalase, superoxide dismutase and glutathione in comparism with caffeine induced group

Result of this study revealed that for Triglycerides (TG) and High density lipoprotein (HDL) there is a significance difference between induced not treated and induced treated when compared with the control group. However, for Total cholesterol (TC) and Low density low protein (LDL), there is a significant difference between the controls when compared with the induced not treated. This result is in agreement with the study of Kamrun*et al*., (2014) who recorded an increase in TC, TG and LDL with a low level of HDL among experimental group.

It is widely accepted that CVD is associated with hypertension and increased levels of low-density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG). In contrast, a low level of high density lipoprotein (HDL) is a risk factor for mortality from CVD (Mora *et al*., 2013).Therefore, the findings from investigations of these parameters may reinforce routine monitoring of hypertensive patients in daily clinical practice to prevent CVD and other harmful consequences of hypertension.

Also, the results showed that there is a significant difference between the controls and induced not treated for Catalase (CAT) and Malondehyde (MDA) while there is no significant difference among all the experimental animals for Super oxide dismutase (SOD) and Glutathione GSH activities.

Reactive oxygen species (ROS) have an important role in the homeostasis of the vascular wall; hence, they could be part of the mechanism that leads to hypertension. Figure 5 shows the effect hypertension on activities of antioxidant marker enzymes of the brain, administration of high dose of caffeine which induced hypertension as seen in Figure 5 brought about increase in the activity of MDA and GSH and lowered the activity of CAT and SOD. The result from this study is supported by research of Nosratola *et al*., (2003) who investigated the effect of lead-induced hypertension of antioxidant markers. Thus, increased ROS production, reduced nitric oxide (NO) levels and reduced antioxidant bioavailability were demonstrated in experimental and human hypertension.

ROS-induced vasoconstriction results from increased intracellular calcium concentration, thereby contributing to the pathogenesis of hypertension. Nifedipine as a calcium channel blocker demonstrated it antioxidative effect by significantly upregulating the reduced activity of brain SOD and MDA brought about as a result of hypertension.

Nifedipine generally demonstrated a positive response against the various physiological effects caused by hypertension induced by high caffeine intake.

CONCLUSION

There is considerable evidence in this study that high dose of caffeine induces blood pressure and also increases body weight among experimental group. The result demonstrated that elevated blood pressure may predict certain disturbances in lipoprotein metabolism as well as bring about ROS generation that disrupts the activities of antioxidant enzyme markers. Meanwhile treatment with nifedipine- a calcium channel blocker class of antihypertensive drug ameliorated the effect of hypertension on the activities of antioxidant enzyme markers and lipid protein metabolism.

REFERENCES

Anjum, R., Zahra, N. and Rehman, K. (2013).Comparative Analysis of Serum Lipid Profile between Normotensive and Hypertensive Pakistani Pregnant Women. .*Journal of Mol.and Gene. Med*. 7(2):64.

Azra, M. and John, F. (2001). Acute Effect of Caffeine on Arterial Stiffness and Aortic Pressure Waveform.*Hypertension*. 38:227–231

Fajobi, A. O., Emma-Okon, B.O., Oyedapo, O.O., Dare, C.A., Akinlalu, A.O., Komolafe, I.J. and Ogunsusi, M. (2020) .Cardio protective activities of *Pterocarpus mildbraedii* leaves on isoproterenol-induced myocardial infarction in rats. *European journal of medicinal plants*. 31(10): 20-37.

Haque, T., Rahman, S., Islam, S., Molla, N. H. and Ali, N. (2019). Assessment of the relationship between serum uric acid and glucose levels in healthy, prediabetic and diabetic individuals. *Diabetology and Metabolic Syndrome*. 11:49.

Islam, A. K and Majumder, A. A. (2012). Hypertension in Bangladesh: a review. *Indian Heart J*. 64(3):319–323.

Kafi, L. A. (2014). A comparative study between olive oil and Nigella Sativa oil in treatment of hyperlipidemia induced in male albino mice. *Iraqi J Vet Med Sci*. 38:123-7.

Kamrun, N. C., Mainuddin, A. M., Mohammad, W. and Sheikh, M. S. (2014). Serum lipid profile and its association with hypertension in Bangladesh.*Vasc Health Risk Manag*. 10: 327–332.

Kamrun, N. C., Mainuddin, A. K. M., Mohammad, W. and Sheikh, M. S. I. (2014).Serum lipid profile and its association with hypertension in Bangladesh.*Vasc Health Risk Manag*. 10: 327–332.

Mora, S., Glynn, R. J. and Ridker, P. M. (2013).High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation.* **128**(11):1189–1197.

Nosratola, D. V., Ching-Yi, L, Farbod, F. and Ram, K. S. (2003).Superoxide dismutase, catalase, glutathione peroxidase and NADPH oxidase in lead-induced hypertension.*Kidney International*. 63(1): 186-194.

Rahman, S., Shiful, I., Tangigul, H., Rahanuma, R. K. and Nurshad, A. (2021). Association between serum liver enzymes and hypertension: a cross sectional study in Bangladeshi adults. *BMC Cardiovascular Disorders*.20:128.

Ramón, R., Matías, L., Felipe, F., and Daniel, H. (2013).Oxidative Stress-Related Biomarkers in Essential Hypertension and Ischemia-Reperfusion Myocardial Damage.*Disease markers*. 35(6): 773-790.

Rodrigo, R.,Bächler, J. P., Araya, J.,Prat, H. and Passalacqua, W. (2007). "Relationship between (Na + K)-ATPase activity, lipid peroxidation and fatty acid profile in erythrocytes of hypertensive and normotensive subjects," *Molecular and Cellular Biochemistry*. 303(12): 73–81.

Saha, M. S., Sana, N. K. and Shaha, R. K. (2006).Serum lipid profile of hypertensive patients in the northern region of Bangladesh.*J Bio-Sci*. 14:93–98.

Shimizu, Y., Kadota, K. and Nakazato, M. (2016).Hemoglobin as a possible biochemical index of hypertension-induced vascular damage.*J PhysiolAnthropol*.35: 4.

Touyz, R. M. (2004). "Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance?" *Hypertension*. 44: 248–252.

Trefor, O. M., Adrianne I. E. and Robert, J. M. (2001).Ace inhibitors, beta-blockers, calcium blockers, and diuretics for the control of systolic hypertension.*American Journal of Hypertension*. 14(3): 241–247.

Wachamo, H. L. (2017) Review on Health Benefit and Risk of Coffee Consumption. *Med Aromat Plants*. 6 (3): 301.