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EVALUATION OF ANTI-HYPERTENSIVE EFFECTS OF *ANDROGRAPHIS PANICULATA* (BURN. F.) WALL EX. NEES EXTRACT ON HEMODYNAMIC PARAMETERS AND REDOX CHANGES IN CADMIUM-INDUCED HYPERTENSIVE RATS

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ABSTRACT

Hypertension, a leading cardiovascular risk factor, can be life threatening if not properly managed. The medicinal plant, *Andrographis paniculata* (AP), has a rich history of ethnobotanical usage among the Yoruba people in south-western Nigeria for the treatment of a wide range of illnesses and complications. However, literature on the antihypertensive effect of the extracts of this plant has not been widely published. Thus in the present study, we investigate the effects of a methanol extract of AP on the blood pressure and biochemical indices of cadmium-induced hypertensive Wistar rats. Systolic, diastolic, and heart rate of the rats were determined. Also, activities of biomarker enzymes [aspartate aminotransferase (AST), total protein (TP), alanine aminotransferase (ALT)], and alkaline phosphatase (ALP), lipid profile [cholesterol (TC), low/very low density lipoprotein (LDL/VLDL), high density lipoprotein (HDL), and triglyceride (TG)], and cardiac antioxidant indices [catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH)] were assessed. The results demonstrated that administration of cadmium chloride ($p < 0.05$) significantly increased the blood pressure, serum biomarkers, and lipid profile with a simultaneous decrease in catalase, SOD, GSH activities, and high-density lipoprotein, which are indicative of oxidative stress. However, treatment with AP extract at varying concentrations resulted in significant decrease in biomarker enzymes and lipid profile, suggesting a protective effect against cardio-toxicity. It was also observed that AP methanol extract significantly increased HDL level and facilitated an increase in catalase, GSH, and SOD activities, an indication of improved antioxidant status. This could be part of the biochemical mechanisms underlying the protective role of AP extract against cardio-toxicity. Therefore, this study provides evidence for the antihypertensive activity of AP and provokes further research for clinical application.

KEYWORD: *Andrographis Paniculata,* Antihypertensive, Cadmium Chloride**,** Lipid Profile.

INTRODUCTION

Elevated blood pressure in the arteries is a chronic medical disorder known as hypertension (HTN), high blood pressure, or arterial hypertension. In the world, it is the most prevalent chronic illness (Marchesi *et al.*, 2018; Carretero and Oparil, 2000). Although there has been no direct identified cause, factors such as sedentary lifestyle, stress, visceral obesity, and potassium deficiency (hypokalemia) are believed to exacerbate the condition (Kyrou., 2006). Moreso, studies have revealed that 85% of hypertension cases occur in patients with a body mass index greater than 25 and, those who experience salt (sodium) sensitivity (Lackland and Egan, 2007; Haslam and James, 2005). The risk of developing hypertension also increases with aging and some inherited genetic mutations (Tuohimaa *et al.*, 2009). A number of oral antihypertensive medications are known, but their usage has been hindered by the associated side effects (Soliman *et al*., 2016).

Therapeutic plants are continuously being studied using animal models in the hopes of creating relatively safe plant-based medicines (Tamiru *et al.*, 2012). *Andrographis paniculata* (Burm.f.) Nees, a member of the Acanthaceae family, is a naturally occurring herbal plant that is endemic in subtropical countries like India, China, Vietnam, Malaysia, Japan, Thailand, and Scandinavia (Jayakumar *et al*., 2012). It is commonly known as the "King of Bitters" and used as a bitter tonic because of its potent bitter flavor (Subramanian *et al*., 2011). However, local variants are found in West Africa, especially in south-west Nigeria (Apata et al., 2024).

Andrographis paniculata plant extracts are rich in flavonoids, tannins, alkaloids, polyphenols, and triterpenoids (Bhan *et al.* 2006). This may serve as the reason for the plant's reported antioxidant, anti-inflammatory, antibacterial, and antihyperglycemic properties (Fardiyah *et al.*, 2020). It is also ethnobotanically used for the treatment of snake bites, scabies, bug bites, skin eruptions, gonorrhoea, bronchitis, malaria, jaundice, diabetes, leprosy, dysentery, anthelmintic, fever, cardiac diseases, and hepatic diseases (Hossain *et al.*, 2014).

In south-west Nigeria, *Andrographis paniculata* is commonly referred to as "Jogbo" and has many applications in Nigerian ethnomedicine, but its potential for the development of pharmaceuticals has not been widely investigated. Thus we investigate the efficacy of *Andrographis paniculata* as a possible candidate for the development of natural medicine for the treatment of hypertension.

MATERIAL AND METHODS

Plant Materials

Fresh plants of *Andrographis paniculata* (AP) were obtained from a private residence located on Latitude $7^0$35 and Longitude $5^0$11 in Ado-Ekiti, Ekiti State, Nigeria. The identification was carried out by Mr. Adejobi of the Department of Crop, Soil, and Pest Management, Federal University of Technology Akure, Nigeria, and it was given the voucher number: FUTA 398.

Extraction of *Andrographis paniculata* and *in-vivo* antihypertensive study

The plant samples were air dried at ambient temperature, and was pulverized. Approximately 500 g of the powdered sample was steeped in 2000 mL of methanol for 72 hours with occasional stirring. A concentrated extract of the plant was obtained using a rotary evaporator and stored in an air-tight container and preserved at 4^0C prior to analysis.

Inducement of hypertension on the Wistar rats was done using the technique of Thijssen et al. (2007). The induced animals were divided into six groups (I–VI), each consisting of five animals, and treated as follows:

Group I (non-hypertensive control): The animals were used as the control and were given distilled water for 14-days.

Group II (hypertensive control): The animals were given 1mg/kg body weight of cadmium chloride only for single administration

Group III (CADM +AP (50 mg/kg)): The animals were given 1mg/kg body weight of cadmium chloride and AP extract (50 mg/kg) by gavage once daily for 14 days.

Group IV (CADM +AP (100mg/kg)): The animals were given 1mg/kg body weight of cadmium chloride and AP extract (100 mg/kg) once daily for 14 days.

Group V (CADM +AP (200 mg/kg)): The animals were given 1mg/kg body weight of cadmium chloride and AP extract (200 mg/kg) once daily for 14 days.

Group VI (CADM +Ram (20mg/kg)): The animals were given 1mg/kg body weight of cadmium chloride and ramiprine (20mg/kg) once daily for 14 days. The dosage was administered according to Oyebami *et al.*, (2017).

Evaluation of Rat Blood Pressure

The rats were placed on a well-padded platform and tightly confined in lateral recumbency. Using an electrosphygnomanometer (CODA, Kent Scientific, USA), indirect blood pressure parameters (systolic, diastolic, and mean blood pressure) were measured by tail plethysmography without the use of anesthesia. After acclimatization, each animal's blood pressure average of at least nine readings taken in a quiescent state was recorded.

Ethical Standard

The animals involved in this study were treated in accordance with the guidelines in the "Guide for the Care and Use of Laboratory Animals," prepared by the National Academy of Sciences and published by the National Institutes of Health. Throughout the experiment, ethical regulations were observed in line with national and institutional standards for animal welfare (PHS, 1996). The study received approval from the scientific committee responsible for overseeing animal research at the Center for Research and Development (CERAD), Federal University of Technology, Akure, Nigeria, with an ethical number of FUTA/ETH/24/152.

Dissection of Animals

The rats were dissected twenty-four hours after the blood pressures were obtained. Their bloods were drawn via heart puncture and placed into EDTA bottles for the purpose of preparing serum and determining biochemical parameters. Using forceps and scissors, we removed the heart and cut off the fatty tissues.

Enzyme Biomarkers

Serum Creatine Kinase (Ck-Mb) activity assay

Assessment of Serum Creatine Kinase (Ck-Mb) levels was assayed according to Stein's (1981) method.

Aspartate Aminotransferase ((AST) assay

Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine as described by Reitman and Frankel (1957).

Aminotransferase (ALT) activity assay

ALT was determined with commercially available test kit in accordance with the manufacturer's instructions (Randox labs, UK) and the premise outlined by Reitman and Frankel (1957).

Activity Assay for Alkaline Phosphatase (ALP)
Serum ALP assay was based on the Englehardt et al., 1970 method, which used commercial assay kits in accordance with the manufacturer's instructions (Randox labs, UK).

ASSAY FOR ANTIOXIDANTS
Catalase Activity Determination
The catalase activity was determined by the method based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate so produced is measured at 570nm – 610nm as described by Sinha (1972).

Determination of Activity of Superoxide Dismutase (SOD)
Superoxide (O^-_2) radical generated by the xanthine oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O^-_2 introduced increased with increasing pH. The level of SOD activity was determined by the method described by Misra and Fridovich (1972).

Determination of the Level of Reduced Glutathione (GSH)
The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable (yellow) color when $5', 5'$ – dithiobis - (2-nitrobenzoic acid, DTNB) (Ellman's reagent) is added to sulfhydryl compounds. The reduced glutathione (GSH) level was estimated using the method described by Beutler *et al*. (1963).

Determination of the Total Protein (TP)
The reaction of cupric ions with protein peptide bonds in an alkaline medium results in the formation of a (blue) complex which exhibits maximum absorbance between 540nm.-570 nm.

Commercially available kits (Randox laboratories, UK) were used to determine the total protein in the serum using the Biuret method as outlined by Weichselbaum (1995).

ANALYSIS OF LIPID PROFILES
Determination of the Level of Total Cholesterol
Cholesterol assay is based on cholesterol esterase hydrolysis of cholesterol esters to form free cholesterol and cholesterol oxidase catalyses the conversion of cholesterol to cholestenone, in which NAD^+ is reduced to NADH, this was determined using commercially available kits as described by Trinder's (1969).

Triglyceride Concentration Assessment
Triglycerides could be determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4- aminophenazone and 4- chlorophenol under the catalytic influence of peroxidase. The level of triglycerides was determined using commercially available kits (Randox laboratories, UK) in accordance with Tietz's (1990) approach.

The cholesterol-high density lipoprotein (HDL-c) assay
Low density lipoprotein (LDL and VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ion. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined. This is estimated in the blood as described by Grove (1979).

Determination of Low Density Lipoprotein (LDL)-Cholesterol
The concentration of low-density lipoprotein in the serum was calculated using the formula of Friedwald *et al.*, (1972) as given below:

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL-cholesterol}$$

Very Low Density Lipoprotein (VLDL) - Cholesterol Determination
The concentration of very low-density lipoprotein in the serum was calculated using the formula of Friedwald *et al.* (1972) as given below:

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{5}$$

Coronary Risk Index Estimation
The coronary risk index was calculated using the formula of Friedwald *et al.* (1972) as given below:

$$\text{CRI} = \frac{\text{Cholesterol}}{\text{High Density Lipoprotein}}$$

Statistical Analysis
Blood pressure and biomarker results were examined using ANOVA version 17.0. the significant difference between the several groups' means. Next were Tukey's tests for mean separation and pairwise comparisons. $P < 0.05$ was established as the cutoff point for statistical significance. Findings.

RESULT AND DISCUSSION
The force of blood pressing against the walls of blood vessels (blood pressure) is based on the heart's cardiac output, or the rate at which blood flows, and the arterioles' resistance to blood flow. In addition to many disorders like heart failure, stroke, heart attack, and renal disease, hypertension can cause harm to organs (Chobanian et al., 2003). To treat and prevent hypertension, various treatment approaches have been used, such as the use of natural compounds produced from plants (Kris-etherton *et al*., 2002). In experimental cardiovascular illness, synthetic antihypertensive drugs like ramipril have been used to lower blood pressure. They have also been shown to increase antioxidant

capability in experimental animals (Mantle *et al*., 2000; Pereira *et al.*, 2004).The current investigation demonstrated that AP methanol extract significantly affects rat blood pressure parameters. Both the systolic and diastolic blood pressures were found to be elevated following cadmium chloride induction, particularly for the non-hypertensive control group (94.00 ± 2.23 mmHg) and the hypertensive control group (173.80± 2.2 mmHg). At all supplied dosages, AP compared well with ramipril and significantly lower the blood pressure parameters. However, AP methanol extract treatment at 200 mg/kg was found to significantly lower blood pressure when compared to the non-hypertensive group and those receiving standard medication (rampril) (figure 1). The various cells, tissues, and organs that make up the body system include biomarkers (enzymes) that, when damaged, seep out and enter the general circulation (Seifter and England, 2012). Serum levels of well-known marker enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatinine kinase (CK) activity, can be measured to identify damage in a cell, tissue, or organ. Serum concentrations of the normal rats' AST and ALT have been shown to increase in illness conditions in a number of investigations (Seifter and England, 2012). This might possibly due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage.

One of the most sensitive indicators used to diagnose organ damage is aspartate aminotransferase (AST), which is abundant in the heart muscle tissues but is also typically seen in the liver, red blood cells, pancreas, and kidney (Sallic *et al.,* 2021). AST, ALT, and ALP activity significantly increased with induced. rats' AST, ALT, and ALP activities were markedly decreased by oral treatment of the methanolic extract of AP (figure 2). This suggests that by preserving the integrity of the plasma membrane and preventing enzymes from leaking through it, the extracts may be able to prevent heart injury. Ramipril and AP extract administration significantly ($P<0.05$) decreased blood AST activity, which may be associated with their cardio-protective effects as shown by heart biomarkers (figure 3). Many disorders that influence bile damage, such as gallstones or tumours that restrict the flow of bile in smaller bile channels inside the liver have higher serum activity of alkaline phosphatase (ALP) (Grant, 2017). Oxidative stress may be the cause of the finding that untreated rats' serum ALP activity was noticeably higher than that of the normal control. The increased alkaline phosphatase level was considerably decreased by pretreatment with ramiprine and extract. This may suggest that the extract did not cause abnormalities in the kidney, liver, or bones—organs that often exhibit high enzyme activity—at the tested dosages. The serum ALT activity significantly increased upon induction of cadmium chloride. When compared to the control, it was shown that animals treated with ramipril and all AP doses had considerably lower levels of alanine aminotransferase. This may suggest that the extract has a protective effect at the tested levels. According to several scientific studies, the antioxidant qualities of certain terpenoids, flavonoids, and steroids protect the heart (Shirwaiker *et al*., 2004).

Therefore, the observed cardioprotective activity may be due to antioxidant phytochemicals in the extracts. Additionally, the current investigation showed that over the course of 14 days, AP extract significantly decreased the rats' serum creatinine activity. The integrity of the cardiac cells' plasma membrane has a direct impact on the serum activity of this cellular enzyme. Therefore, the extract may have inhibited the release of this enzyme into the serum by preserving the integrity of the heart membrane, which in turn prevented the enzyme from leaking into the serum (Chen *et al*., 2008).

After being exposed to cadmium chloride toxicity, the heart's low-density lipoprotein, triglyceride, and cholesterol levels negatively increased while its high-density lipoprotein levels decreased (figure 4). This was a sign of oxidative stress brought on by the production of free radicals. The results of this investigation showed that AP extract significantly mitigated the elevated serum lipid profile, which included lower high density lipoprotein, triglycerides, cholesterol, and low-density lipoprotein compared to the untreated mice. The results demonstrated that, in comparison to the control group, ramipril and *Adrographis paniculata* extract significantly decreased TC, TG, and LDL-C at all dosages while increasing HDL.

CONCLUSION

The results in the present study revealed that *Adrographis paniculata* extract could be a very good therapeutic agent for the treatment of hypertension. Absence of toxicity in the in-vitro bioassay to the heart and extra-cardiac tissues of the experimental rats at the evaluated dosages (200 mg/kg) attest to its safety for use in medicine. The results also showed a dose-dependent antihypertensive activity. The investigation suggested that *Adrographis paniculata* extract may exhibits a significant antihypertensive and antioxidant activities, providing a potential therapeutic strategy for the treatment of hypertension using plant-derived medicine.

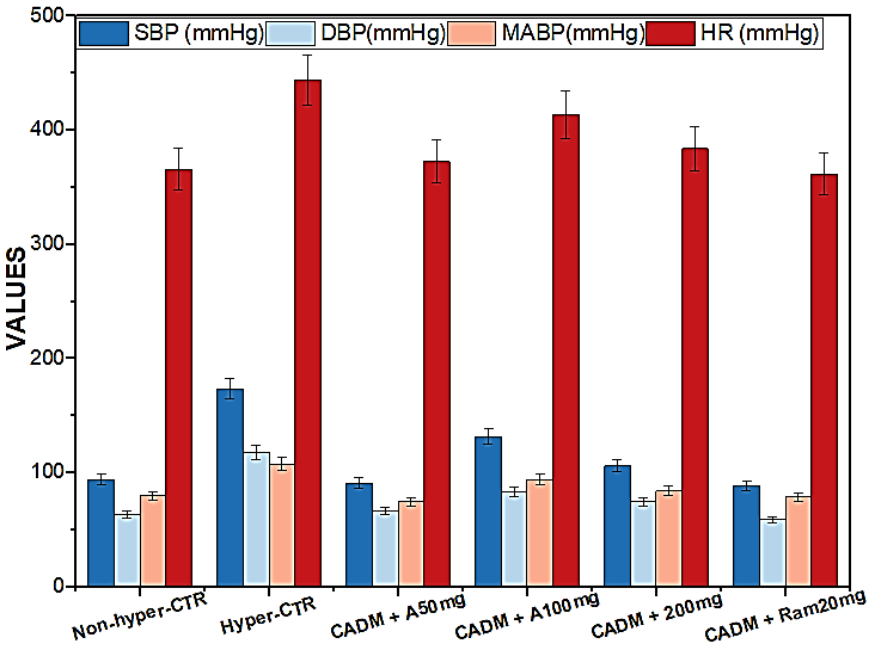

Figure 1: Effects of *Andrographic paniculata* methanol extract on hemodynamic parameters in Cadmium-induced hypertension in rats.

Key
SBP – Systolic blood pressure;
DBP – Diastolic blood pressure;
MABP- Mean arterial blood pressure;
HR – Heart rate.

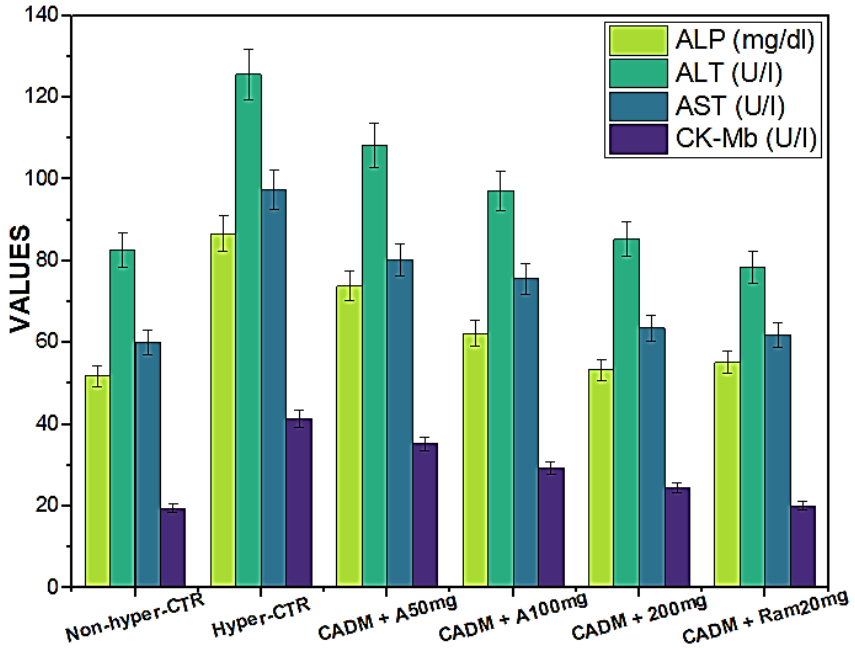

Figure 2: Effect of *Andrographic paniculata* methanol extract on Serum Biomarkers on Cadmium-induced hypertension in rats.

KEY
ALP = Alkaline phosphatase
ALT = Alanine amino transferase
AST = Aspartate amino transferase
CK = Creatinine Kinase
LDH = Lactate Dehydrogenase

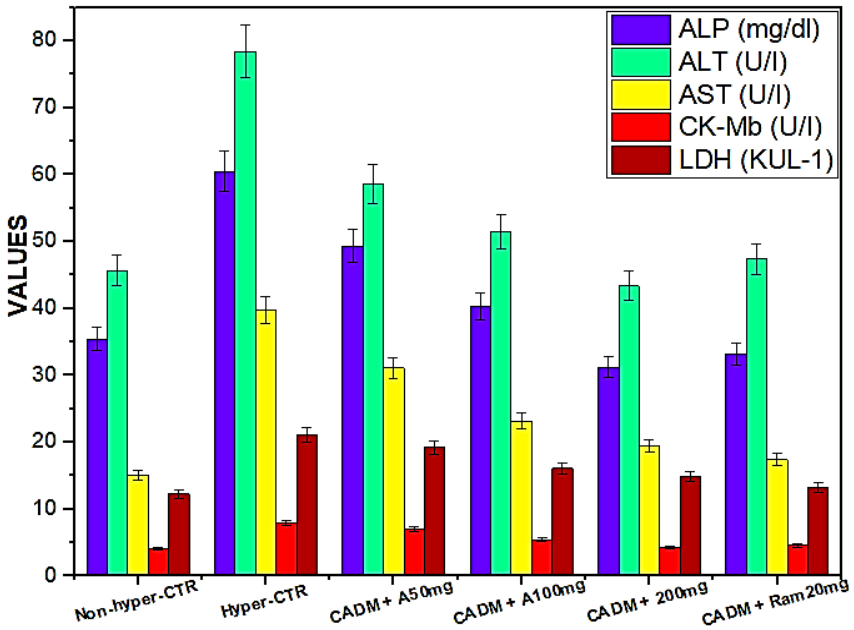

Figure 3: Effect of *Andrographic paniculata* methanol extract on Heart Biomarkers in Cadmium-induced hypertension in rats.

KEY
ALP = Alkaline phosphatase
ALT = Alanine amino transferase
AST = Aspartate amino transferase
CK = Creatinine Kinase
LDH=Lactate Dehydrogenase

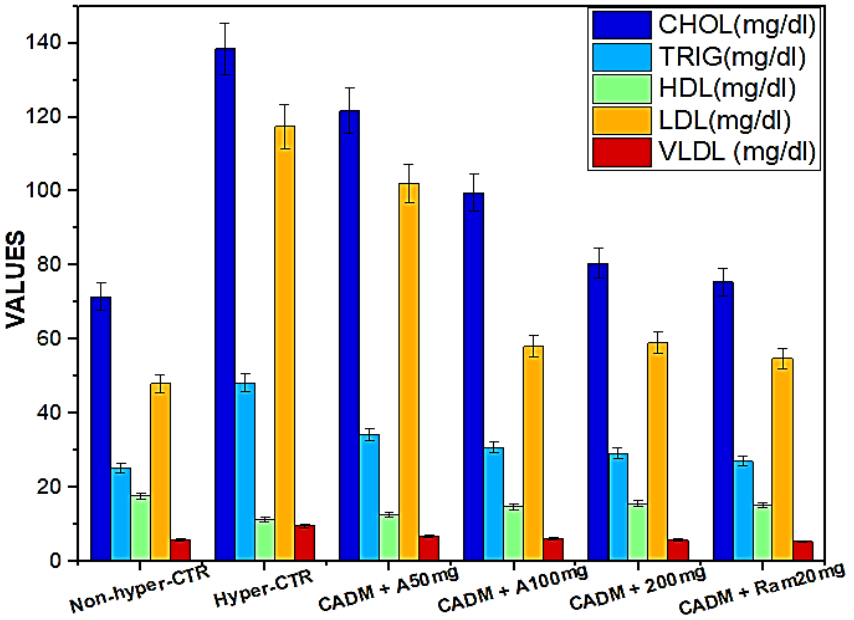

Figure 4: Effect of *Andrographic paniculata* methanol extract on Serum Lipid Profile in Cadmium-induced hypertension in rats.

KEY
CHOL–Cholesterol;
LDL -Low density lipoprotein;
TG-triglyceride;
VLDL-Very low density lipoprotein;
HDL-High density lipoprotein

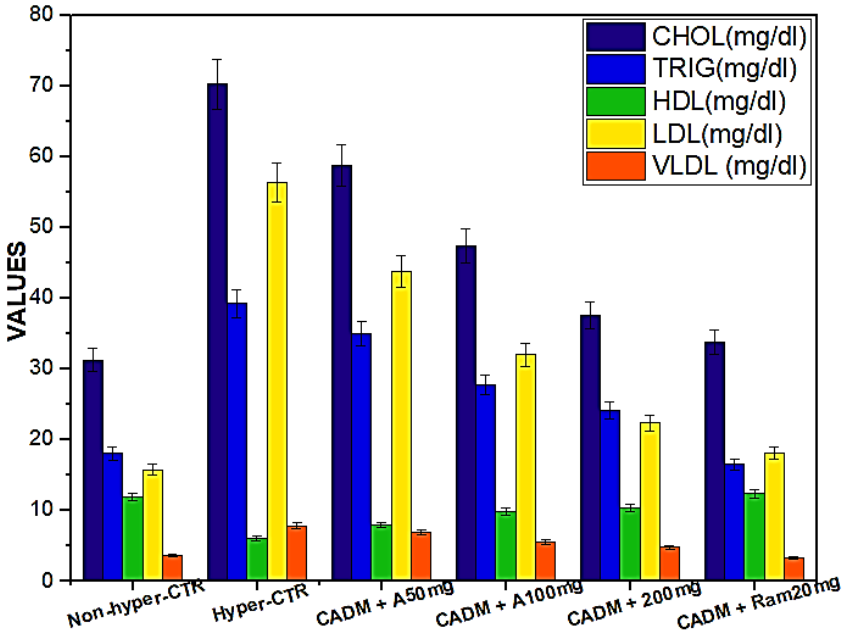

Figure 5: Effect of *Andrographic paniculata* methanol extract on Heart Lipid Profile in Cadmium-induced hypertension in rats.

KEY
CHOL– Cholesterol;
LDL - Low density lipoprotein;
TG- triglyceride;
VLDL- Very low density lipoprotein;
HDL- High density lipoprotein.

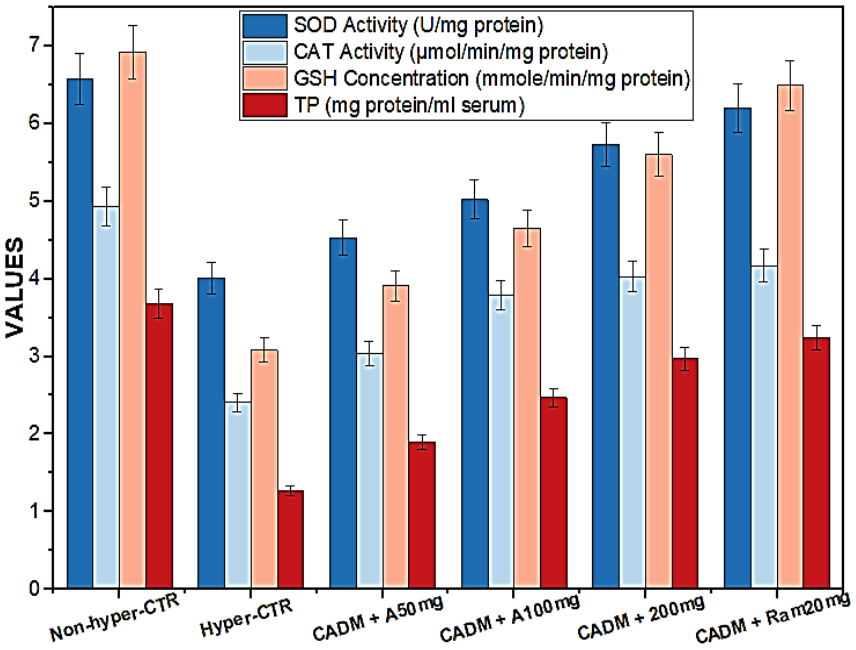

Figure 6: Effect of *Andrographic paniculata* Plant extract on Heart Antioxidant Enzymes in Cadmium-induced hypertension in rats

KEY
SOD = Superoxide dismutase
CAT = Catalase
GSH = Reduced Glutathione
TP = Total Prote

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