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Effect of n-hexane extract of baobab (*Adansonia digitata*) fruit on biochemical parameters of l-n^{g}-nitro arginine methyl ester induced hypertension in rats

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L-N^{G}-Nitro arginine methyl ester (L-NAME) was used to induce hypertension in this experiment and the intervention plant extract was *Adansonia digitata* fruit. A total of 30 rats were used for this research and divided into 6 groups of 5 animals each. Group 1 was control (water and feed *ad libidum)*, group 2 (L-NAME), group 3 (ramipril 10 mg/kg), group 4 (*A. digitata* 200 mg/kg), group 5 (*A. digitata* 400 mg/kg) and group 6 (*A. digitata* 400 mg/kg no induction). Extract administration lasted 21 days after which animals were anesthetized and blood samples taken for analyses. Prior to this, recordings of blood pressure and weights were recorded. The fruits extract of the baobab plant was found to significantly reduce (P<0.05) the blood pressure in the hypertensive animals compared to the control and test drug. The Na and Cl concentrations in blood were significantly reduced compared to the control and L-NAME group at dose of 400 mg/kg. It was also found to prevent hyperkalemia and normalized creatinine as well as serum protein levels. The extract also significantly reduced the body weight of the animals at same dose. In conclusion, the extract reduced blood pressure in this experiment by reduction of Na (salt) concentration. It is a promising plant that will help sufferers from hypertension. Further studies are needed to study about molecular mechanisms involved in its activities.

Key words: L-name, adansonia digitata, hypertension, Nacl, antioxidant.

INTRODUCTION

Hypertension is a chronic medical condition characterized by sustained elevation in arterial blood pressure. High blood pressure remains one of the strongest singular risk factor for cerebrovascular accidents, coronary artery diseases and kidney injuries (Rehab et al., 2017). Hypertension is a pandemic that affects over 600 million people globally, and is responsible for 13% of deaths worldwide. It ranks third in terms of disability-adjusted-life-years and it has been estimated that by 2025, 20% of the world's adults will be hypertensive (Ramanathan and Thekkumalai, 2019).

L-N^{G}-Nitro arginine methyl ester (L-NAME) is an L-arginine analogue and is a principal agent of nitricoxide synthase (NOS) with variable influence on blood pressure (Blanc et al., 1999; Jana et al., 2012). Several studies have shown that short term administration of L- NAME

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in experimental animals result in a sharp rise in arterial blood pressure especially by its inhibitory effect on NOS (Ayse et al., 2012; Zeliha et al., 2015). The acute inhibition of nitric oxide (NO) synthesis by L-NAME causes increase in vascular smooth muscle tone and sympathetic discharges via a neurogenic mechanism which results in an elevated blood pressure (Xavier et al., 2000; Thewarid et al., 2015).

Baobab (*Adansonia digitata*) is a majestic tree indigenous to Africa. Every part of the tree has variable medicinal and nutritional value. It has been used in managements of ailments like diarrhea, malaria and various microbial diseases. The fruits of *A. digitata* are ten times richer than oranges in vitamin C content making this plant a potent antioxidant (Kamatou et al., 2012; Sami et al., 2019). It has also been found to have hepatoprotective effect in CCL4-induced hepatotoxicity in rats (Musab et al., 2015).

Therapeutic effects of a varying range of herbal formulas in L-NAME induced hypertension have been reported in a number of researches (Vishal et al., 2012). Works using the baobab fruit for same purpose are unavailable. The present study seeks to find the effect of the baobab fruit extracts on biochemical parameters that could be affected by L-NAME induced hypertension.

MATERIALS AND METHODS

Plant collection and identification

The sample used for this research were fresh and matured Baobab fruit purchased from a local market in Kaduna, which were identified and authenticated in the herbarium unit of Biological Science, Benue State University Makurdi, Nigeria and samples were collected after identification and kept at herbarium unit.

Preparation of baobab fruit

The baobab fruit were washed thoroughly with clean water to reduce the microbial load and other contaminants that might adhere to the baobab fruit. The baobab fruit were chopped into smaller sizes with a clean knife and air dried at room temperature. They were pulverized using a laboratory mechanical grinder and the fine powder obtained was stored until needed. 825 g was extracted with 1 L of N-Hexane (via maceration) for 48 h. The mixture was decanted and filtered using Whatman No. 1 filter paper. The filtrate was concentrated to dryness using a water bath at 50°C, giving a dark-brown paste with a percentage yield of 10%. The extract was later reconstituted such that 1 g of the paste was dissolved in 10 ml of normal saline to make up the concentrations of the stock solution which was labeled appropriately and refrigerated at 4°C until required for use.

Phytochemical analysis

The N-Hexane extract of *A. digitata* fruit was subjected to phytochemical screening test to detect the presence or absence of flavonoids, tannins, alkaloids, saponins and glycosides(Table 1 and 2). Also, proximate analysis was done to determine the presence or absence of moisture, protein, crude fibres, ash, fats and oil and Carbohydrates (Table 3). The phytochemical analysis and proximate analysis were done at the laboratory of the Chemistry Department, Benue State University, Makurdi. The Standard method of Harbone (1983) was used in the phytochemical determination. Proximate analysis was determined according to the method of Association of Official Analytical Chemist (AOAC, 1990).

Five grams of each powdered extract were soaked in 100 ml N-Hexane for 48 h. 110 mm Whatman filter paper was used to filter the solution. The extract was subsequently evaporated to adequate weight using Ohaus water bath and dried to adequate weight in hot air oven at 40°C. The extract was used immediately for phytochemical and proximate analysis.

Experimental animals

A total of 30 adult male Sprague Dawley rats weighing 200 to 270 g were used for the experiment, they were purchased from the animal house unit, College of Health Sciences, Benue State University, Makurdi. The animals were housed in wooden cages and were provided with growers mash produced by grand cereals oil and mills limited, Jos Plateau State, Nigeria. Clean water *ad libitum* was provided. The animals were maintained under standard laboratory condition (25°C) with relative humidity of 62 to 73% under a 12 h light dark cycle (ethical approval ID: ABU.LABRESEARCH.08381). They were acclimatized for 14 days prior to the experiment.

Induction of hypertension

Hypertension was induced by oral administration of 40 mg/kg L-NAME in the rat after their base line body weight and blood pressure were measured. The blood pressure of the rats was measured daily until sustained hypertension was attained using non-invasive, tail-cuff blood pressure meter by PANLAB (NIBP; LE5001).

Experimental design

Thirty male rats after acclimatization were used in the current study. Their basal blood pressure and body weight were recorded before the commencement of the experiment which lasted for 21 days. After 1 week acclimatization and hypertension induction using L-NAME the rats were randomly divided into the following experimental groups.

Group A (n-5) Normotensive (control group)
Group B (n-5) Hypertensive no treatment
Group C (n-5) Hypertensive + Rampril 10 mg/kg
Group D (n-5) Hypertensive + *A. digitata* 200 mg/kg
Group E (n-5) Hypertensive + *A. digitata* 400 mg/kg
Group F (n-5) Normotensive + 400 mg/kg *A. digitata* (why you used the high concentration dose and not the lower dose or used 200 and 400 mg

Determination of body weight

The body weights of the experimental rats were determined prior to the commencement of the experiment and at the end of the experiment. Their body weights were taken using a digital top loading weighing scale (XY3002C).

Determination of blood pressure

The non-invasive blood pressure meter equipment was used for the

Table 1. Qualitative result of phytochemical screening of *A. digitata* fruit.

Parameter	Ethanol or Hexane	Water
Alkaloids	+	+
Tanin	-	-
Flavonoid	+	-
Glycoside	+	+
Saponin	+	+
Terpenoid	+	-
Phenol	-	-

The *A. digitata* fruit contains alkaloids, flavinoids, glycoside, saponin and terpinoid

Table 2. Quantitative results of phytochemical screening of *A. digitata* fruit.

Parameter	Value (%)
Alkaloid	1.33
Flavonoid	4.21
Glucoside	0.32
Saponin	1.93
Terpenoid	1.14

The *A. digitata* fruit contains high concentration of flavinoids compared to other phytochemicals.

Table 3. Result of proximate analysis of *A. digitata* fruit.

Parameter	Value (%)
Moisture	2.31
Protein	19.3
Crude Fibre	9.4
Ash	1.6
Fat and Oil	12.1
Carbohydrate	76.1

The *A. digitata* fruit is rich in carbohydrate, proteins and fats.

systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurement. Rats were held in a restrainer on a preheated platform with the tail exposed and a hand towel was used to cover the restrainer to keep the rat calm. The tail was massaged gently to encourage blood circulation to the tail, the occlusion cuff and a volume pressure-recording cuff were placed close to the base of the tail. The digital values for the systolic and diastolic blood pressure (SBP) were recorded. All measurements were taken by the same person in the same quiet environment at the same time in the morning daily.

Blood collection

Rats were sacrificed after being anaesthetized with ethyl ether at the end of 21 days. Blood was collected by cardiac puncture into plan tubes, after clotting the blood was dislodged and centrifuge at 1200 rpm for 5 min using bench centrifuge. The serum was collected and used immediately for the biochemical test.

Biochemical analysis

Serum potassium, sodium, chloride, carbonate, urea, creatinine, phosphate, uric acid, albumin, calcium and total protein were determined using clinical chemistry kit (ERBA Diagnostics Mannlelin GrusH, German) and Spectrumlab 23A (Gulfex Medical & Scientific England) according to the manufacturers specification.

Statistical analysis

Data obtained from the study were expressed as mean ± SEM. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons using SPSS statistical tool version 20. Values of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

This study showed that *A. digitata* has a potent antihypertensive action. The 400 mg/kg dose reduced the L-NAME induced blood pressure better than the control drug (Figure 1). Doses of 200 and 400 mg/kg of the fruit extract was found to significantly reduce the serum concentrations of sodium and chloride ion levels compared to control and L-NAME induced group ((Figure 2). Concomitant reduction of sodium and chloride (NaCl) is a relevant pathway for reduction in blood pressure as it possesses a strong correlation with development of hypertension (Theodore, 2005; Miguel et al., 2019). Water retention by NaCl is known to worsen hypertension (McGregor, 1986). Also, a newer theory shows that NaCl in elevated levels causes high Na-evoked cerebrospinal fluid volume and secondary increase in sympathetic nerve activity which triggers vasoconstriction and ultimately hypertension (Mordecai et al., 2012). Fidele et al. (2020) demonstrated that aqueous extract of *A. digitata* stem bark (100 and 200 mg/kg) significantly reduced systolic, diastolic and mean arterial blood pressures in L-NAME induced hypertensive rats. Their proposed mechanisms were corrections of dyslipidemia and partial nitric oxide bioavailability. The present studies

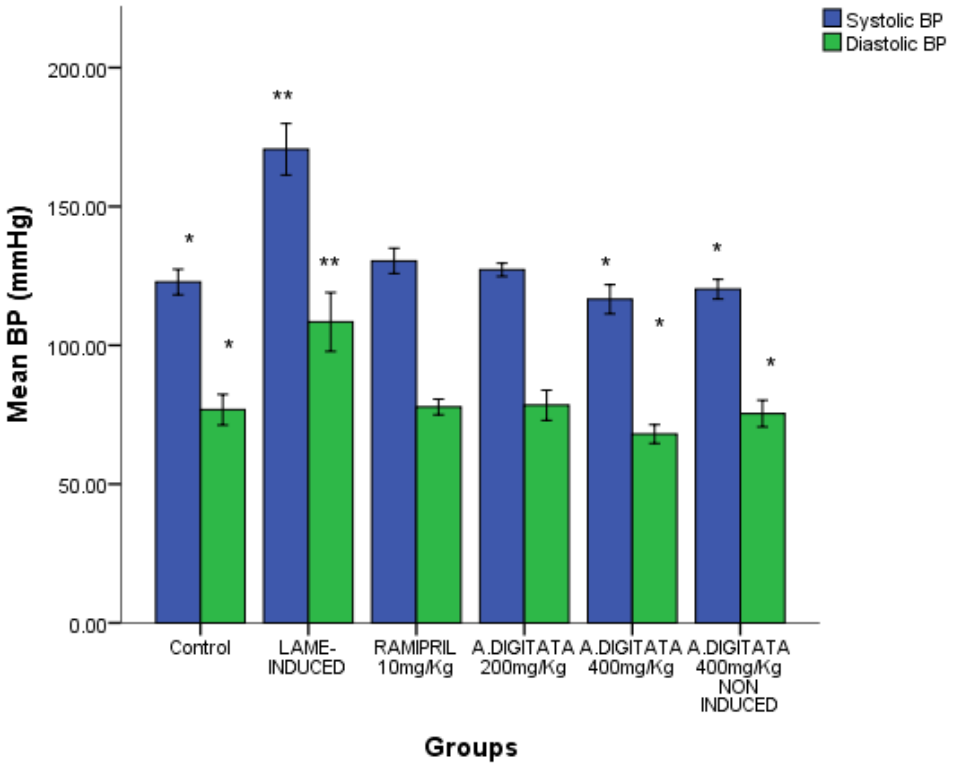

Figure 1. L-NAME significantly elevated the blood pressure (BP) of the mice compare to control. The treated group (400 mg/kg) significantly reduced the BP to normal. *Indicate significant reduction compared to **p<0.05.

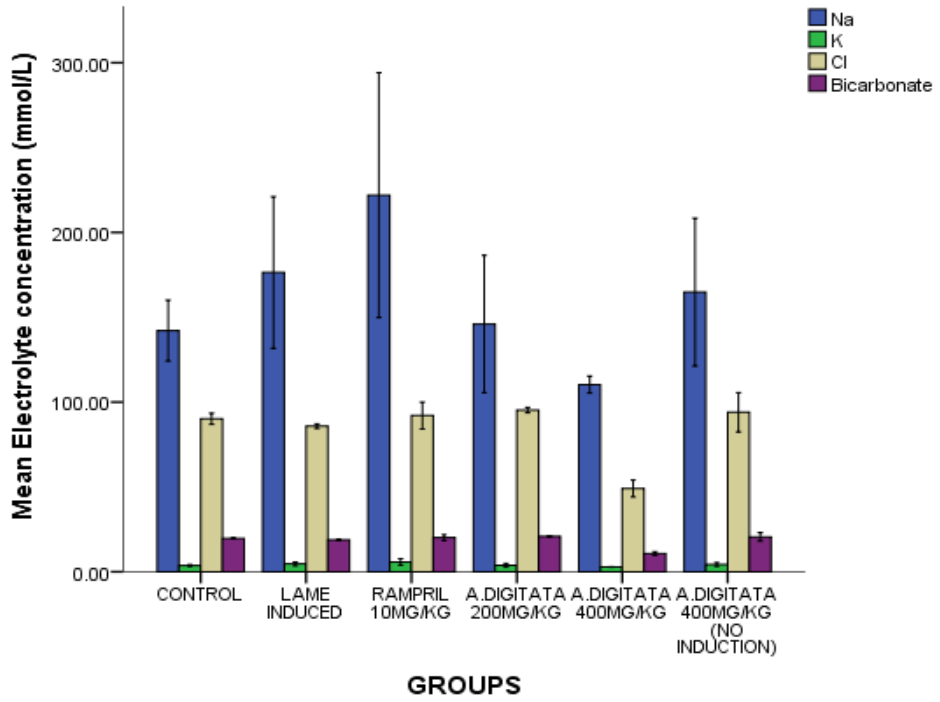

Figure 2. The *A. digitata* at 400 mg/kg significantly reduced (P<0.05) serum concentrations of potassium, sodium, chloride and bicarbonate compared to the other groups. Only sodium concentration was reduced significantly at 200 mg/kg.

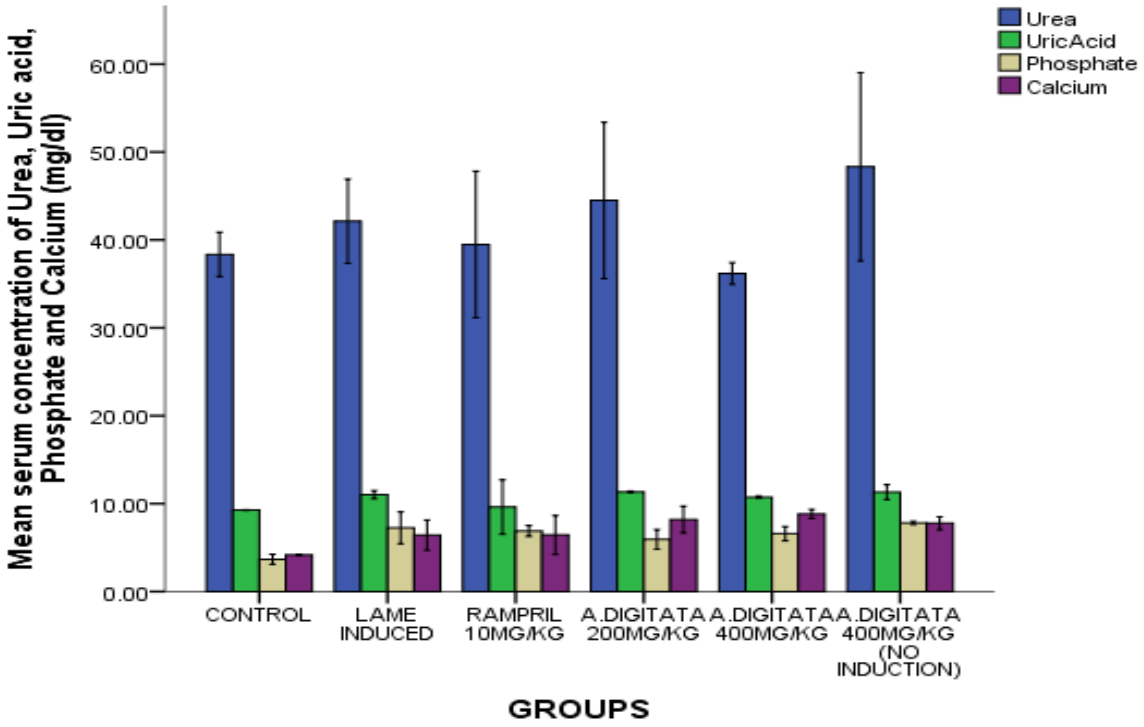

Figure 3. The *A. digitata* fruit significantly increased (P<0.05) serum calcium oncentration at 200 and 400 mg/kg and phosphate levels at all doses. It also caused a significant increase in urea levels only at 400 mg/kg but had no effect on uric acid levels in all doses.

however proved clearly for the first time that *A. digitata* fruits extracts can normalize blood pressure comparable to standard drugs via the reductions in serum NaCl levels. Another important finding is the renoprotective action of *A. digitata* by reduction of serum potassium levels to normal levels compared to the elevated levels seen in L-NAME groupv ((Figure 1). Hyperkalemia has been strongly linked to kidney injuries, which is further worsened by reduced GFR, poor excretion of the ion, and evolution of other cardiovacscular co-morbidities (Renato, 2020). Similarly, 400 mg/kg of *A. digitata* was found to significantly reduce serum creatinine levels which were elevated significantly in the L-NAME group (((Figure 4)). Creatinine is an important marker for testing renal function; it is a breakdown product of creatinine phosphate in muscle. When elevated it may indicate a renal dysfunction and it is (Josef et al., 2001; Shivaraj et al., 2010). It is known that elevated serum creatinine is associated with kidney disease and strongly related to inappropriate blood pressure management. It can be proposed therefore that *A. digitata* is both cardio and renoprotective. A recent finding by Hayat et al. (2020) corroborated our findings as they demonstrated low serum creatinine levels using *A. digitata* fruit extracts (400 mg/kg) in metabolic syndrome Wistar rats. The present study however did not find any effect of the extract on serum urea, uric acid and phosphate levels. The extract however maintained normal serum calcium level which was found to be reduced by the test drug – ramipril ((Figure 3). *A. digitata* also maintained a normal total serum protein levels comparable to control levels ((Figure 5). Matawalli et al. (2004) showed that aqueous leaf extract of *A. digitata* caused hypoalbuminemia. This contradicts our study as the fruit extract used normalized serum albumin and total protein levels. The only study that tends to corroborate our studies was by Sola-Ojo et al. (2016) that demonstrated a stable serum protein levels though using the seed extract in broilers unlike the fruit extract we used in rats. In this study also the serum calcium levels were significantly higher in the fruit extract groups. This could be as a result of the rich concentration of calcium found in the fruit. Jitin et al. (2015) reported a calcium level of 293 mg/100 g; which is equivalent to milk of 125 mg/100 mg. Finally, in this study 200 mg/kg *A. digitata* significantly reduced the body weight of the rats compared to other doses, the test group and the control group ((Figure 6). This finding is supported by a recent study that showed that fruit extracts of *A. digitata* can cause weight reduction dose dependently (Hayat et al., 2020). On the contrary, a study by Ogunleye et al. (2019) demonstrated significant weight gain with the fruit extracts in rats at doses of 40, 80 and 160 mg/kg, respectively. These doses are lower when compared with the present study showing that higher doses of the

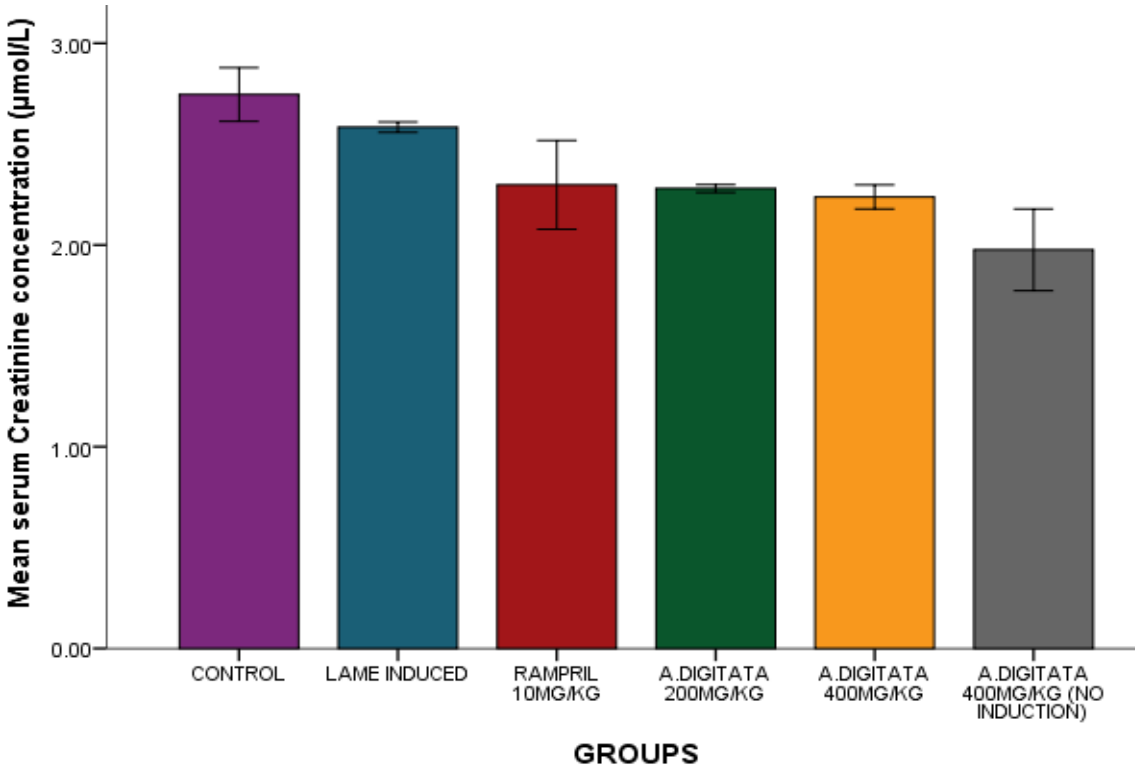

Figure 4. *A. digitata* fruit significantly reduced (P<0.05) serum concentration of creatinine at 400mg/kg compared to the control and L-NAME groups.

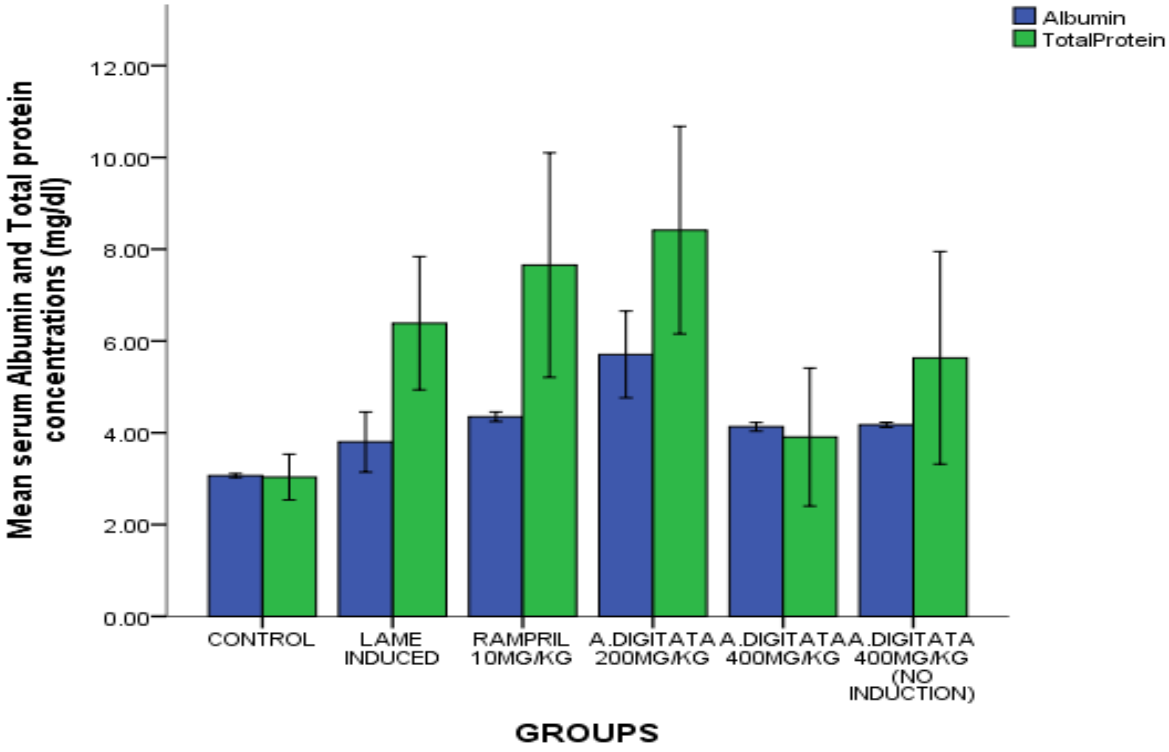

Figure 5. *A. digitata* fruit significantly reduced (P<0.05) total serum protein at 400 mg/dl compared ramipril but significantly increased serum albumin at all doses compared to control group.

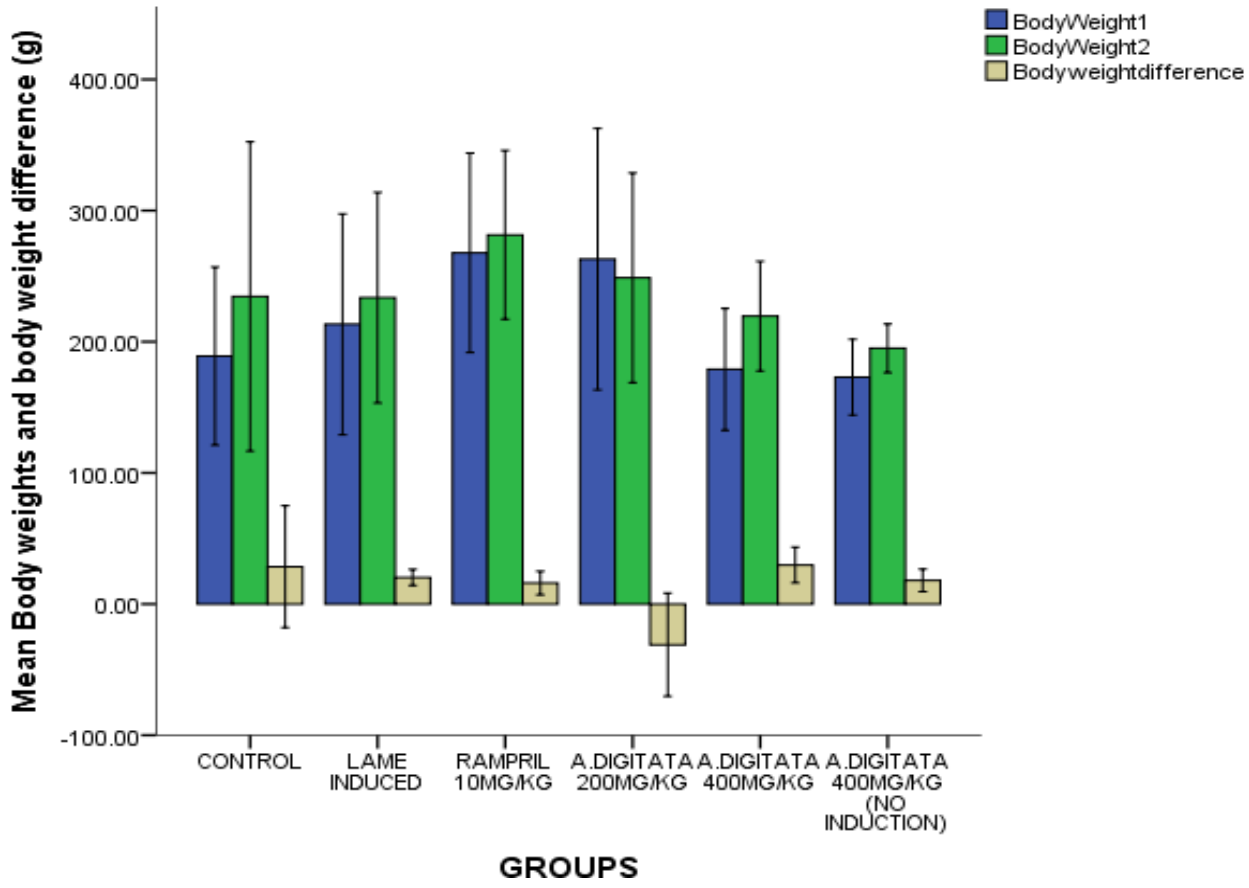

Figure 6. *A. digitata* at 200 mg/kg caused a significant (P<0.05) decrease in body weight compared to other groups.

extract are more useful in achieving weight loss. The actions of this essential fruit extract can also be associated with its rich antioxidant (vitamin C, phenols and flavinoids) composition which helps in ameliorating oxidative stress (Selamoglu, 2017).

Conclusion

This study demonstrated antihypertensive action as well as amelioration of the negative effects of elevated blood pressure using the fruit extracts of *A. digitata* in L-NAME-induced hypertension model. The dose of 400 mg/kg seems to be more effective in reducing blood pressure by the salt (NaCl) reduction pathway. The fruit extract was also found to be both cardio and reno-protective by preventing hyperkalemia and rise in creatinine levels. It also shows promise in management of obesity as it is found to significantly reduce body weight of the animals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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