LASU Journal of Medical Sciences
Official Publication of the Faculty of Basic Medical Sciences and Faculty of Basic Clinical Sciences
Lagos State University College of Medicine, Ikeja
www.lasucom.edu.org.
E-mail: lasujms@lasucom.edu.ng

Research Article

Aqueous extract of *Parkia biglobosa* ameliorates risk markers of cardiometabolic diseases in spironolactone treated and high-salt fed Sprague-Dawley male rats.

Oyabambi Adewumi O[1*], Akinnuga Akinjide M[2,3], Areola Emmanuel D[1], Echibiri Success E[1], Imam-Fulani Aminat O[1], Abdulsalam Abdulbasit O[1], Oluranti Olufemi I[4]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. [2]Department of Human Physiology, Faculty of Basic Medical Sciences, University of Cross River State, Okuku Campus, Yala, Cross River, Nigeria. [3]Department of Human Physiology, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa. [4]Department of Physiology, College of Health Sciences, Bowen University, Iwo, Nigeria.

[*]***Author for Correspondence:***
Oyabambi A. O.
Email: oyabambi.ao@unilorin.edu.ng; +2348032826203

Keywords:
Parkia biglobosa, High-salt diet, Spironolactone, Cardiometabolic diseases, Sprague-Dawley rats.

ABSTRACT

Objectives: The number of people with salt-sensitive hypertension and cardiometabolic diseases (CMD) are increasing due to high-salt diet (HSD) consumption globally. *Parkia biglobosa* (PB), an African locust bean tree, has been reported to have several cardiovascular protective properties but its ameliorative effects on CMD are scarcely reported. Therefore, this study aimed at investigating the effects of PB stem bark aqueous extract on some risk markers of CMD in weanling male rats subjected to HSD and Spironolactone (Sp) treatment.

Methods: Twenty-five weanling male rats (95-105 g) were divided into 5 groups: Group 1 (Control); Group 2 (untreated HSD) fed on normal chow and HSD (8% NaCl); Group 3 (HSD+Sp); Group 4 (HSD+PB); Group 5 (HSD+Sp+PB) fed on HSD (8% NaCl) and received either 80 mg/kg of Sp or 400 mg/kg of PB and both as treatment, respectively. After 6 weeks of treatment, blood andcardiac samples were collected from each animal for biochemical analysis.

Results: Administration of both PB and Sp or only PB, significantly decreased the plasma or cardiac adenosine deaminase (8.39 vs18.61 mg/dl; $P< 0.05$), xanthine oxidase (0.30 vs 0.70 mg/dl; $P<0.05$), C-reactive protein(30.07 vs 66.88 mg/dl; $P<0.05$), lipids (except high-density lipoprotein), uric acid (5.93 vs 8.51mg/dl; $p< 0.05$), sodium (122.60 vs141.38 mmol/l;$P<0.05$), and potassium (5.57 vs 7.83mmol/l; $P<0.05$) concentrations. Contrarily, the plasma, as well as cardiac nitric oxide and endothelial nitric oxide synthase, increased significantly by the same treatment (8.83 vs 4.48mg/100 mg tissue; $P<0.05$).

Conclusion: *Parkia biglobosa* or its administration with Spironolactone ameliorates associated-risk markers of cardiometabolic disease which are triggered by a high salt diet.

INTRODUCTION

With salt being a major preservative of food before refrigeration, the amount of salt in several foodstuffs have significantly increased and eating habits which include salt consumption have substantially changed in industrialized countries.[1] Therefore, the numbers of people affected by salt-sensitive hypertension and cardiometabolic diseases including heart attack, stroke, diabetes, insulin resistance and non-alcoholic fatty liver disease are growing.[1,2]

High-salt diet (HSD) is a risk factor that has detrimental effects on the tissues and organs of cardiovascular systems.[3,4] HSD alters the metabolic functions of the body system via increase in plasma sodium concentration and expansion of extracellular volume which subsequently lead to increased blood pressure.[4,5] Also, several researches have shown that incessant intake of HSD may result in dysregulations of the cardiovascular systems, leading to fluid overload, inflammation, elevated serum triglyceride and cholesterol, decreased vascular bioavailability of nitric oxide (NO) which cause several endothelial and metabolic dysfunctions in the blood vessels and heart.[6-9]Moreover, studies have shown that HSD triggers elevated uric acid (hyperuricaemia) which is already known as an independent risk factor of cardiometabolic diseases.[10,11] In addition, increased activity of adenosine deaminase (ADA) and xanthine oxidase (XO) can be hypothesized to result in hyperuricaemia. However, the association between HSD and these enzymes (ADA and XO) of uric acid metabolism has not been well documented. Studies have shown that both HSD and hyperuricaemia are associated with impairment of nitric oxide (NO) production due to inhibition of endothelial nitric oxide synthase (eNOS).[12,13] The human body prevents the risk of developing cardiometabolic dysfunctions and tolerates

excessive salt intake for a short time. However, dietary salt reduction and pharmacological interventions are the therapeutic approaches to cardiometabolic diseases, but studies have shown that these approaches are with side effects in certain individuals.[14-16] Therefore, other therapeutic approaches that prevent or manage cardiometabolic dysfunctions due to HSD are necessary. As a result, the quest for other approaches has presented traditional alternative medicine as an effective, safe, and inexpensive therapy.

Parkia biglobosa (PB) is a multipurpose fodder tree that belongs to the family Mimosaceae, popularly called the "African locust bean tree". PB is found in many countries, especially in the West African coast where the seeds are fermented into locust beans (known as "iru" in Yoruba, "daddawa" in Hausa, and "ogiriokpe" in Igbo), a popular local food seasoning known to be rich in protein and vitamin B2. The seed, leaf, and stem bark extracts of PB have been shown to exhibit hypotensive activities by decreasing blood pressure via vasodilation.[17,18] Apart from the reported hypotensive activities of PB, there is scarce information on the effects of the aqueous extract of PB stem bark in relations to other risk factors or markers of cardiometabolic functions. Therefore, this study was designed to investigate the effects of the aqueous extract of PB stem bark on some risk factors or markers which are associated with cardiometabolic diseases in weanling Sprague-Dawley male rats subjected to HSD.

METHODS

Plant material and extract preparation

The stem barks of *Parkia biglobosa* (PB) were collected in the University of Ilorin, College of Health Sciences premises, and air-dried in a shade and was identified at the herbarium of the Department of Plant Biology with voucher number UILH|001|2019|1016. The phytochemical contents (Sample label and Serial number: MTL/14920/009) of the stem bark were analysed at the Molecular Toxicology Laboratory, Deprtment of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria as described in Table 1.

Table 1: Phytochemical contents of *Parkia biglobosa* stem bark

Parameter Measured	Value Obtained
Alkaloid	5.297 ± 0.334 mgAE/g
Tannin	5.517 ± 0.885 mgGAE/g
Phenol	129.443 ± 3.311 mgGAE/g
Flavonoid	30.667 ± 4.225 mgQUE/g

mgAE/g= milligrams ascorbic acid equivalent per gramme of sample
mgGAE/g= milligrams Gallic acid equivalent per gramme of sample
mgQUE/g= milligrams Quercetin acid equivalent per gramme of sample

The air-dried stem barks were ground into powder using a milling machine and then sieved. For conventional extraction, 5 g of the powdered stem bark was soaked in 50 mL of distilled water in a beaker for 48hours and stirred intermittently every 2-3 hours to mix properly. This process was repeated until the desired amount of residue was obtained. At the end of the 48 hours, the solution was filtered with a Whitman filter paper (125 mm) to separate the residue from the filtrate. Thereafter, the filtrate was filtered and concentrated into a powdery form via water bath at 40°C, and 400 mg/kg was administered as the dose of the aqueous extract according to Tijani *et al*.[19]

A gas chromatography-mass spectrometer (GC-MS) was used to separate the chemical mixture and analyzed different chemical constituents of the aqueous extract of PB stem bark as described in Table 2 and GC-MS trace in Figure 1.

Table 2: Gas chromatography and Mass spectrometer

Chemical constituent	Resident time (RT)	% Area concentration	Peak height
Dodecanoic acid	13.384	5.85	11539468
	13.835	5.90	14955791
	14.708	7.90	20141357
	14.933	6.29	20697431
	15.187	6.97	19511014
	15.694	5.40	16003563
	16.004	6.23	14774676
	16.483	8.26	13336278
	16.736	5.03	13877632
	16.849	1.54	12732811
	17.018	5.98	15093299
	17.299	4.84	12706034
	17.638	2.74	7272968
	18.004	2.42	5501031
	18.342	1.71	4905644
	18.736	0.91	1347430
1, 2, 3-Propanetriy l ester	14.933	6.29	20697431
	15.187	6.97	19511014
	16.483	8.26	13336278
	16.736	5.03	13877632
	17.018	5.98	15093299
	17.638	2.74	7272968
	18.004	2.42	5501031
	18.736	0.91	1347430

A GC-MS was used for the instrumental analysis of stem bark of *Parkia biglobosa* extract which showed main compounds peaking at different times, dodecanoic acid and 1,2,3-propanetriol ester at various resident times (RT).

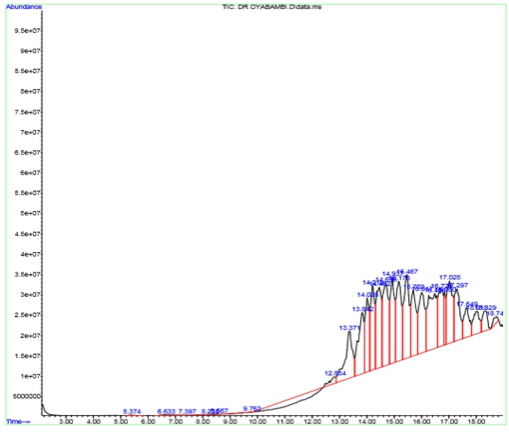

Figure 1: Extract characterization showing qualitative information on the active constituents of back stem of *Parkia biglobosa* and the peaks identified

Experimental animals

Twenty-five (25) weanling Sprague-Dawley male rats that weighed between 95-105 g were obtained from the animal house of the College of Health Sciences, University of Ilorin, Nigeria. The animals were acclimatized for 2 weeks and maintained under standard environmental conditions of temperature (22-26^{0}C), relative humidity (50-60%) and 12-hour dark/light cycle. The study was carried out according to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and was approved by the Ethical Review Committee of the University of Ilorin (UERC) with ethical number UERC/ASN/2019/357.

Treatment and experimental design

Spironolactone (Sp), a reference drug, was purchased from Pfizer, B25796 and manufactured by Piramal Healthcare (UK). It was administered at a dose of 80 mg/kg according to Sica.[14] After 2 weeks of acclimatization, the weanling animals were randomly assigned into five groups (n = 5), fed on normal chow or high-salt diet (HSD, 8% NaCl) as described by Sofola *et al*.[20] and received either Sp or PB extract and both as treatment for 6 weeks as follows:

Group 1 (Control): normal chow and 2 mL of distilled water (vehicle).

Group 2 (Untreated HSD): HSD (8% NaCl) and 2 mL of distilled water (vehicle).

Group 3 (HSD+Sp): HSD (8% NaCl) and Sp (80 mg/kg) as treatment.

Group 4 (HSD+PB): HSD (8% NaCl) and PB aqueous extract (400 mg/kg) as treatment.

Group 5 (HSD+Sp+PB): HSD (8% NaCl) and both Sp and PB aqueous extract as treatment.

Blood sample collection and tissue harvesting

At the end of the treatment period, the animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). While the animals were unconscious, blood samples were collected by cardiac puncture into heparinized tubes. The heart was then excised from eachanimal, rinsed in normal saline, weighed, and corrected to body weight to eliminate variability. The blood samples were centrifuged at 3000 rpm for 15 min to obtain plasma, and the plasma was stored frozen until needed for biochemical assay. Thereafter, 100 mg of the heart tissues were homogenized in cold phosphate buffer solution by a homogenizer and subsequently centrifuged at 10000 rpm for 10 min at 40^{o}C.

Determination of relative weight

The relative weights of the heart of the animals were determined from the percentage of the ratio of the cardiac weight to the body weight i.e.

Relative weight = cardiac weight / body weight × 100

Biochemical Analysis

The biochemical analysis of sodium, potassium, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), nitric oxide (NO), endothelial nitric oxide synthase (eNOS), uric acid (UA), c-reactive protein (CRP), xanthine oxidase (XO), adenosine deaminase (ADA) concentrations was determined via standardized enzymatic colorimetric methods by using specific assay kits as described in the instruction manuals of the manufacturer (Fortress diagnostics, Antrim, UK). The LDL was estimated by Friedewald's formula (21), LDL = Total cholesterol - (HDL + Triglyceride/5).

Statistical Analysis

All data were expressed as means ± SEM. Data was analyzed with SPSS, version 23 statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair-wise comparisons of mean values among the groups. Statistically significant differences were accepted at $p<0.05$.

RESULTS

Effect of *Parkia biglobosa* on relative heart weight, mean body weight, food and water intakes in spironolactone treated and high-salt fed male rats

The relative heart weight of the untreated HSD-fed rats and HSD-fed rats that received Sp treatment was significantly increased when compared to that of the control group and HSD-fed rats that received PB extract or both PB and Sp, as indicated in Table 3. However, administration of both PB extract and Sp in the presence of HSD significantly decreased the relative heart weight when compared to the control group and HSD-fed rats that received PB extract only.

Effects of *Parkia biglobosa* on plasma sodium and potassium concentrations in spironolactone treated and high-salt fed male rats

There was a significant increase in plasma sodium concentration in the untreated HSD-fed rats (HSD Group) when compared to the control group ($p<0.05$). On the contrary, administration of only Sp, PB extract or both significantly decreased the plasma sodium concentration when compared to the untreated HSD-fed rats but not significantly different in comparison to the control group (Figure 2 A). As shown in Figure 2 B, administration of only Sp caused a significant increase in plasma potassium concentration in comparison to the untreated HSD-fed rats and the control group. However, administration of only PB extract or both PB extract and Sp significantly decreased the plasma potassium concentration when compared to the administration of Sp alone ($p<0.05$).

Table 3: Effect of *Parkia biglobosa* on relative heart weight, mean body weight, food and water intakes in spironolactone treated and high-salt fed male rats

	CTR	HSD	HSD+Sp	HSD+PB	HSD+Sp+PB
Relative heart weight (%)	0.62±0.05	0.75±0.04*	0.86±0.18*	0.64±0.05#	0.55 ± 0.13#
Body weight(g)					
Initial	101.00 ± 4.39	100.13±4.07	100.30 ± 3.04	102.00 ± 3.29	103.20 ± 1.80
Final	150.50±8.84	182. 90±3.20*	166.50 ±3.14#1	63.85 ± 7.43#	145.50 ± 2.60#
%Change	49.01±0.40	82.66 ± 0.20*	66.00 ± 0.70*#	60.64 ± 0.10*#	40.99 ± 0.50#β
Food Intake(g/kg/day)					
Initial	83.40 ± 2.30	82.40 ± 1.30	82.20 ± 1.20	81.57 ± 3.10	84.40 ± 1.40
Final	100.90 ± 2.50	110.40 ± 2.10	104.51 ± 2.40	103.20 ± 1.50	103.40 ± 2.40
%Change	20.98 ± 2.10	33.98 ± 2.20*	27.14 ± 2.10#	26.52 ± 1.15#	22.51 ± 1.30#
Water Intake(ml/kg/day)					
Initial	50.90 ± 4.10	55.60 ± 3.20	52.60 ± 4.50	53.40 ± 2.60	51.30 ± 5.30
Final	55.70 ± 5.30	85.70 ± 6.60*	65.30 ± 5.50	70.00 ± 5.90	61.20 ± 6.10#
%Change	9.43 ± 2.20	54.13 ± 3.10*	24.14 ± 2.30*#	31.09 ± 5.92*#	19.29 ± 3.70*#

n = 5 rats per group; *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group).

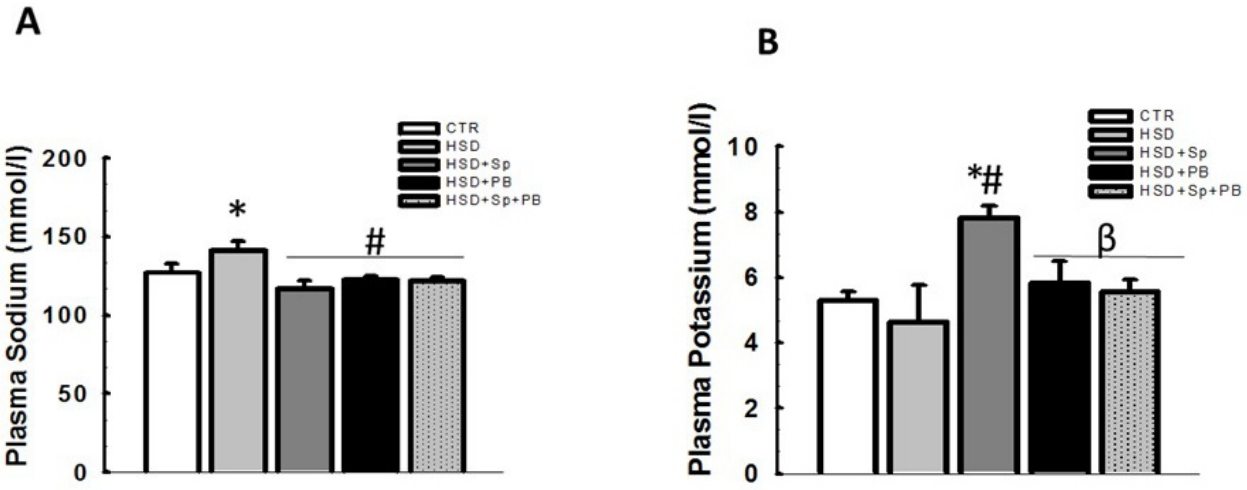

Figure 2:Effects of *Parkia biglobosa* extract on plasma sodium (A) and potassium (B) in HSD-fed male rats; n = 5 rats per group, *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group)

Effects of *Parkia biglobosa* on plasma lipid profiles in spironolactone treated and high-salt fed male rats

In Figure 3 A, Figure 3 B, and Figure 3 C respectively, the plasma concentrations of triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) were significantly increased in the untreated HSD-fed rats and HSD-fed rats with Sp administration when compared to the control group. On the other hand, only PB extract or both PB extract and Sp significantly decreased the plasma triglyceride, total cholesterol, and low-density lipoprotein in comparison to the untreated HSD-fed rats and HSD-fed rats with Sp administration only. Moreover, the HDL plasma concentration significantly decreased in the untreated HSD-fed rats and HSD-fed rats with Sp administration when compared to the control group (Figure 3 D). On the contrary, administration of PB extract or both PB extract and Sp significantly increased the plasma HDL concentration in comparison to the untreated HSD-fed rats.

Effects of *Parkia biglobosa* on plasma and cardiac uric acids in spironolactone treated and high-salt fed male rats

The plasma and cardiac uric acid levels of untreated HSD-fed rats and HSD-fed rats with Sp administration (HSD+Sp) were significantly increased when compared to the control group (p<0.05), as indicated in Figure 4 A and 4 B. Only PB extract or its combination with Sp significantly decreased the plasma uric acid level in comparison to the untreated HSD-fed rats. However, administration of only PB extract significantly decreased the cardiac uric acid concentration in comparison to the untreated HSD-fed rats (Figure 4 B).

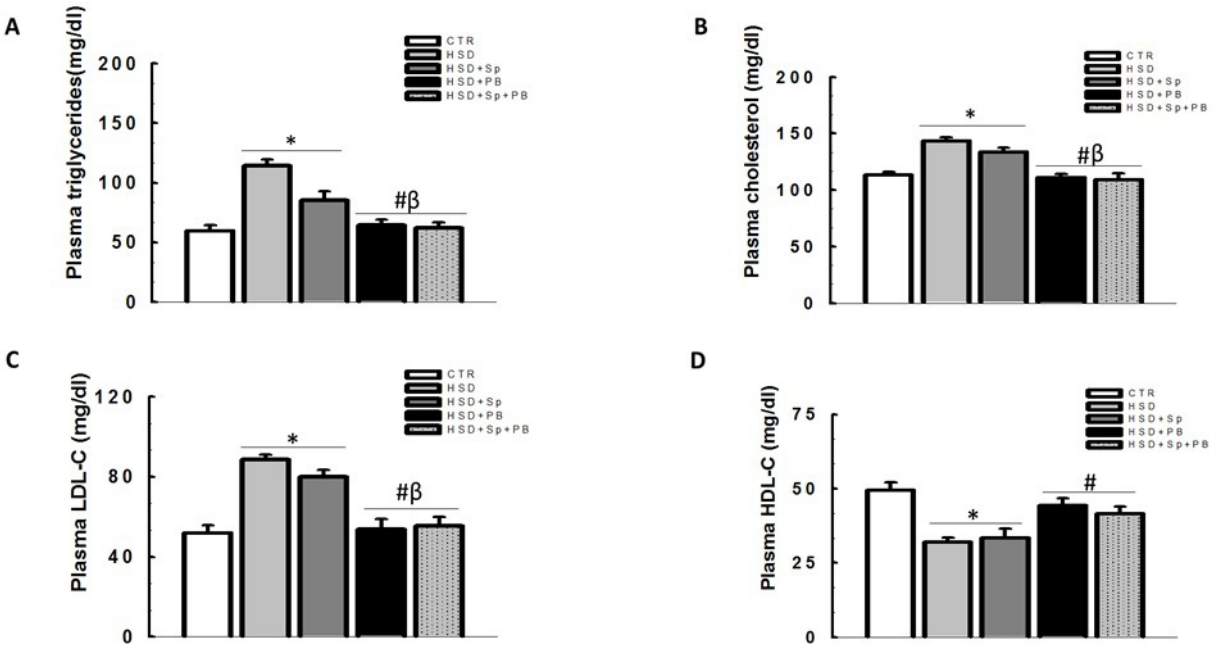

Figure 3: Effects of *Parkia biglobosa* extract on plasma triglyceride (A), total cholesterol (B), low-density lipoprotein, LDL (C) and high-density lipoprotein, HDL (D) in HSD-fed male rats;n = 5 rats per group, *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group)

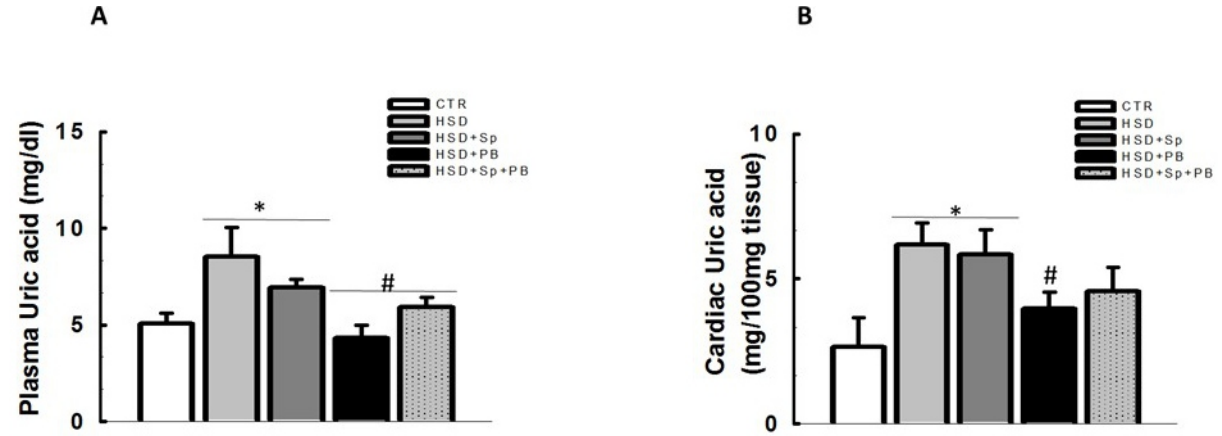

Figure 4: Effects of *Parkia biglobosa* extract on plasma uric acid (A) and cardiac uric acid (B) in HSD-fed male rats; n = 5 rats per group, *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group)

Effects of *Parkia biglobosa* on plasma and cardiac adenosine deaminase (ADA) and xanthine oxidase (XO) in spironolactone treated and high-salt fed male rats

The plasma and cardiac activities of ADA were significantly increased in the untreated HSD-fed rats when compared to the control group (Figure 5 A and 5 B respectively). On the other hand, administration of the combination of both PB extract and Sp significantly decreased the plasma activities of ADA in HSD-fed rats when compared to the untreated HSD-fed rats (HSD group), HSD-fed rats with Sp (HSD+Sp), and HSD-fed rats with PB extract (HSD+PB). In addition, the administration of only PB extract or its combination with Sp significantly decreased the cardiac activities of ADA in HSD-fed rats in comparison to the untreated HSD-fed rats.

Moreover, the plasma and cardiac activities of XO were significantly increased in the untreated HSD-fed rats when compared to the control group (Figure 5 C and Figure 5 D respectively). However, administration of only PB extract or both PB extract and Sp significantly decreased the plasma and cardiac activities of XO when compared to the untreated HSD-fed rats, as shown in Figure 5 C and Figure 5 D.

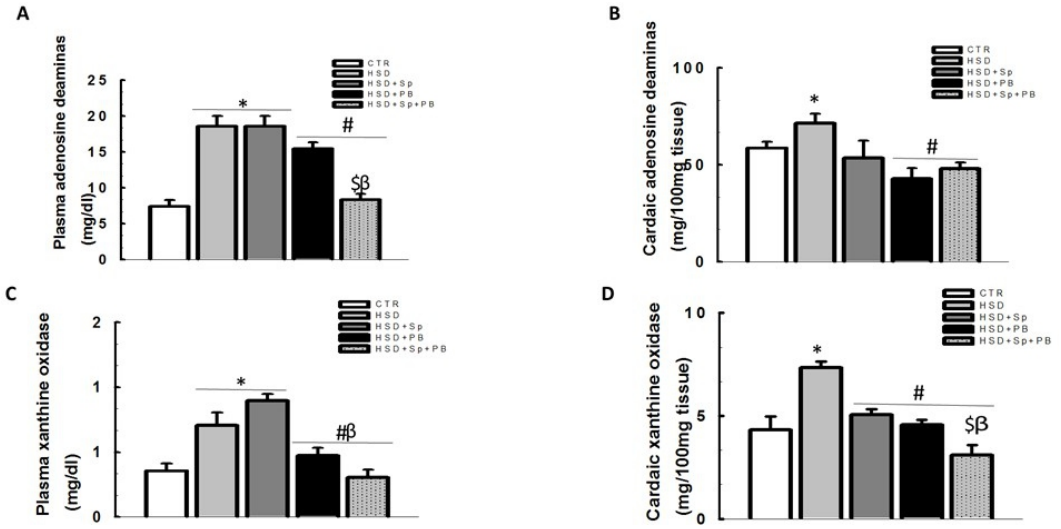

Figure 5: Effects of *Parkia biglobosa* extract on plasma adenosine deaminase (A), cardiac adenosine deaminase (B), plasma xanthine oxidase (C) and cardiac xanthine oxidase (D) in HSD-fed male rats, *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group),

Effects of *Parkia biglobosa* on plasma and cardiac nitric oxide (NO) and endothelial nitric oxide synthase (eNOS) in spironolactone treated and high-salt fed male rats

The plasma and cardiac concentrations of NO were significantly decreased in the untreated HSD-fed rats and HSD-fed rats that received Sp administration alone when compared to the control group (Figure 6 A and Figure 6 B respectively). However, administration of only PB extract and its combination with Sp significantly increased the plasma and cardiac NO concentrations in the HSD-fed rats when compared to the untreated HSD-fed rats. Furthermore, the plasma and cardiac concentrations of eNOS were significantly decreased in the untreated HSD-fed rats when compared to the control group, as indicated in Figure 6 C and Figure 6 D respectively. On the other hand, the plasma and cardiac eNOS concentrations were significantly increased in the HSD-fed rats that received PB extract alone or both PB extract andSp when compared to the untreated HSD-fed rats.

Effects of *Parkia biglobosa* on plasma and cardiac on C-reactive protein (CRP) in spironolactone treated and high-salt fed male rats

The plasma and cardiac concentrations of CRP in untreated HSD-fed rats and HSD-fed rats with Sp administration increased significantly when compared to the control group (Figure 7A and 7 B). On the contrary, administration of only PB extract, as well as its combination with Sp significantly decreased the plasma and cardiac concentrations of CRP in comparison to the untreated HSD and HSD+Sp groups.

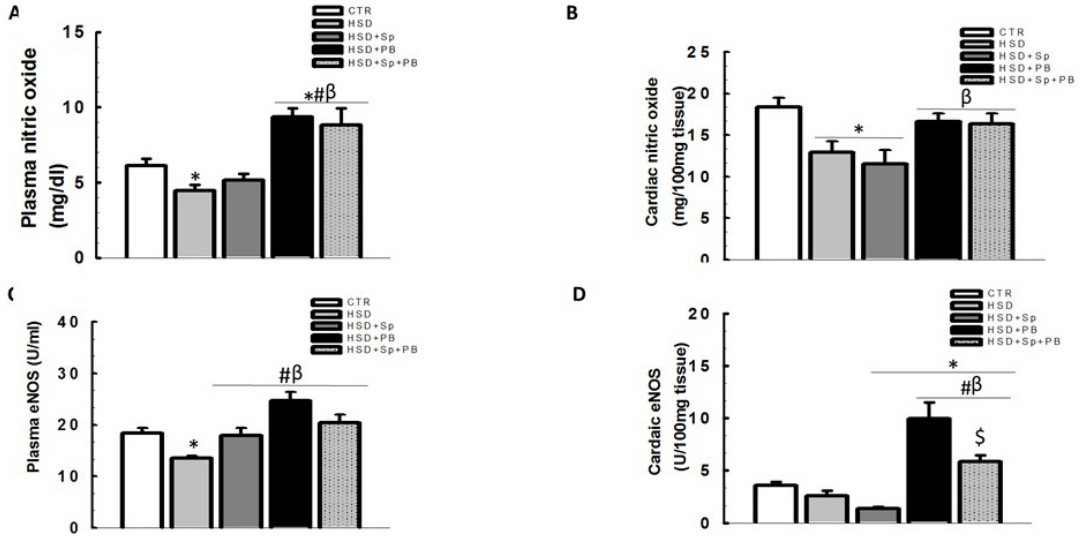

Figure 6: Effects of *Parkia biglobosa* extract on plasma nitric oxide (A), cardiac nitric oxide (B) plasma endothelial nitric oxide synthase, eNOS (C), and cardiac eNOS (D) in HSD-fed male rats;n = 5 rats per group, *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group), $p<0.05 (vs HSD+PB group)

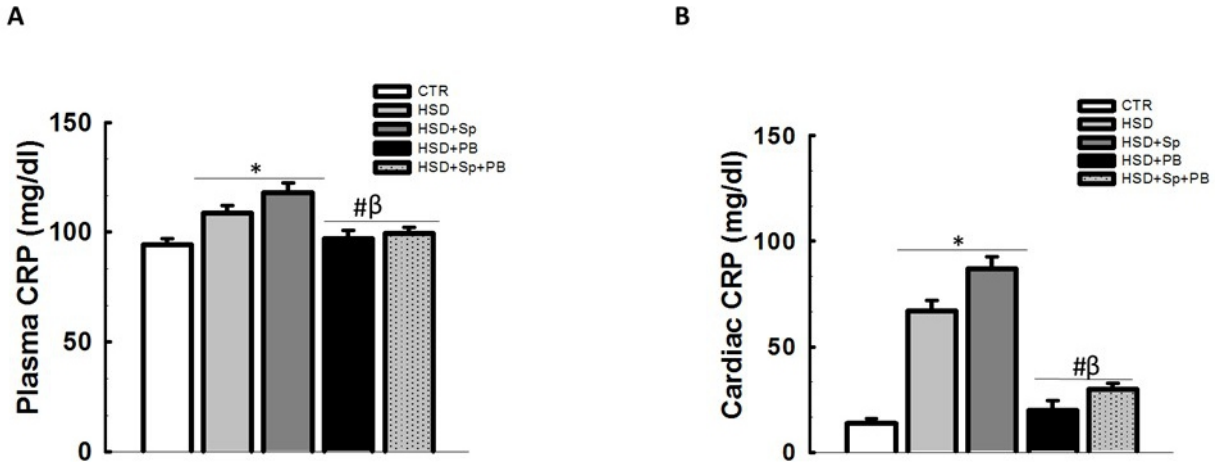

Figure 7: Effects of *Parkia biglobosa* extract on plasma C-reactive protein, CRP (A) and cardiac CRP (B) in HSD-fed male rats;n = 5 rats per group, *p<0.05 (vs control group), #p<0.05 (vs HSD group), βp<0.05 (vs HSD+Sp group).

DISCUSSION

This study investigated the adverse effects of high salt intake as well as the plausible therapeutic effects of the stem bark of *Parkia biglobosa* (PB) aqueous extract or Spironolactone (Sp), and both on markers of cardiometabolic functions. Previous experimental studies have shown that the hydroalcoholic extract of PB stem bark caused vasorelaxation and the aqueous extract of the bark decreased the blood pressure.[2,23] However, the present study showed that the aqueous extract of PB stem bark ameliorates cardiometabolic dysfunctions in high-salt diet. High-salt diet (HSD) has been reported to cause some cardiometabolic changes such as electrolytes imbalance (especially sodium and potassium imbalance), dyslipidaemia, cardiac dysfunctions and inflammatory responses.[4,5]

In this study, HSD caused a significant increase in plasma sodium and a slight but insignificant decrease in potassium concentration. Increased plasma sodium concentration has been known to elicit deleterious cardiometabolic changes which may involve activation of mineralocorticoid receptors. Spironolactone (Sp) is a mineralocorticoid blocking agent that competes with the cytoplasmic aldosterone receptor.[14] Its mechanism of action is tocompetitively block the epithelial and non-epithelial actions of aldosterone in the distal tubule of the nephron, thus, preventing sodium and water retention, and causing potassium retention. It is not surprising that the administration of Sp ameliorated plasma concentrations of sodium and caused potassium retention in HSD-fed animals. This observation is consistent with other studies.[1,14] The potassium-retention effect of Sp in this study was significantly increased in HSD-fed animals and this showed that Sp acted as a potassium-sparing diuretic. Therefore, Sp can cause deleterious elevation of circulating potassium as an adverse effect if used for therapy in salt-induced cardiometabolic disease. On the other hand, administrations of either only PB extract or both PB extract and Sp normalized the sodium and potassium plasma concentrations in HSD-fed animals. Therefore, it is reasonable to suggest that PB extract has a mineralocorticoid blocking action since the administration of only PB extract normalized plasma sodium and does not spare potassium as inSp administration. We, therefore, infer that the combination of PB extract and Sp prevented the deleterious increase in potassium level when Sp was administered alone. Thus, PB may be appropriate for co-administration with Sp (as a diuretic) to prevent the potassium-retention effect of Sp.

Moreover, HSD caused a significant decrease in plasma concentration of high-density lipoprotein and a significant increase in plasma concentrations of triglycerides, low-density lipoprotein, and total cholesterol, thus, showing overtdyslipidaemia. Dyslipidaemia has been reported in different studies in association with metabolic syndrome, cardiometabolic dysfunction, and salt-induced hypertension.[9,24] In addition, the mechanism by which HSD increases triglycerides is still controversial but it is well known that high salt intake impairs lipid metabolism.[9] Interestingly, administration of Sp to HSD-fed animals did not normalize dyslipidaemia.

However, administration of only PB extract or both PB extract and Sp ameliorated lipid profile in HSD-fed animals. Evidence in previous studies has shown that the aqueous extract of PB stem bark has anti-lipidaemic effects due to its component called tannin which reduces hypertriglyceridaemia and hypercholesterolaemia.[25-27] The anti-lipidaemic effects of PB have also been attributed to its hypolipidaemic component called saponins.[28] Therefore, the observed anti-lipidaemic effects of PB in this study agreed with other previous studies.[25-28]

Furthermore, CRP is a general marker of inflammation and a risk marker for cardiovascular diseases.CRP plasma concentration increases whenever there is tissue damage in the body due to inflammation.[29] Therefore, the significant increase in the plasma and cardiac CRP in both the untreated HSD-fed rats and HSD-fed rats that received Sp indicated that there was the occurrence of systemic and cardiac inflammation in both groups. On the other hand, the PB extract caused a significant decrease in the plasma and cardiac CRP in the HSD-fed animals. Hence, this suggests that PB stem bark has anti-inflammatory properties as reported in the findings of Kouadio et al and Nwaehujor *et al*.[30,31]

In this study, HSD disrupts the endothelial function by

a decrease in the plasma and cardiac concentrations of nitric oxide (NO) and endothelial nitric oxide synthase (eNOS). In previous studies, reduction in NO is strongly associated with increased levels of reactive oxygen species (ROS) which are generated by NADPH oxidase, xanthine oxidase (XO) or uncoupled eNOS within the vascular wall.[32,33] These previous studies were corroborated with the observed increase in the cardiac XO and profound reduction of NO and eNOS in the plasma and cardiac tissues of the untreated HSD-fed rats. However, administration of PB extract ameliorated the endothelial function by a significant increase in NO and eNOS. Therefore, we suggest that since PB stem bark has been reported to have antioxidant and anti-inflammatory properties, the production of ROS that disrupts NO and eNOS functions was reduced in HSD-fed rats that received either PB extract or both PB extract and Sp. Similarly, the enhancement of endothelial function via NO production because of the antioxidant property of PB is supported by other studies.[17]

Furthermore, uric acid is known to cause endothelial dysfunction, vascular smooth muscle cell proliferation, increased IL-6 synthesis, and impairment of nitric oxide production, all of which contribute to the progression of cardiometabolic diseases.[34-36] The administration of PB extract or its combination with Sp showed a significant decrease in plasma uric acid level with a concurrent decrease in ADA and XO. It is expected that a decrease in uric acid levels should be accompanied by a decrease in both ADA and XO. This probably suggests that the observed uric acid lowering properties of the PB extractare mediated through the ADA/XO/UA pathway.

In conclusion, PB stem bark ameliorated risk markers of cardiometabolic diseases in HSD and Sp treated rats via its antioxidant, anti-inflammatory, anti-lipidaemic, and uricosuric effects. In addition, these effects might be due to the presence of its secondary metabolites such as tannin, saponin, alkaloid, flavonoid, and phenol which have been reported to possess cardioprotective properties. The enhanced electrolyte balance, endothelial function-enhancing activities, and UA lowering capacity of PB in this study is worthy of note. Therefore, further studies are needed on the exact mechanisms by which PB exhibits its ameliorative effects in the cardiometabolic diseases model.

Conflict of Interest

The authors declared no known conflicting interests that could affect the outcome of this work.

ACKNOWLEDGMENTS

The authors appreciate the moral and technical support from the HOPE Cardiometabolic Research Team of the Department of Physiology, University Ilorin, Nigeria

REFERENCES

1. Agócs R, Sugár D, Szabó AJ. Is too much salt harmful? Yes blood pressure dietary approaches to stop hypertension trial of hypertension prevention. Pediatr Nephrol, 2020;35(9):1777–1785.
2. Bibbins-Domingo K, Chertow GM, Coxson PG, Moran A, Lightwood JM, Pletcher MJ *et al*. Projected effect of dietary salt reductions on future cardiovascular disease. N Engl J Med, 2010; 362(7): 590–599.
3. Farquhar WB, Edwards DG, Jurkovitz CT, Weintraub WS. Dietary sodium and health: More than just blood pressure. J Am CollCardiol,2015;65(10):1042-1050.
4. Robinson AT, Edwards DG, Farquhar WB. The influence of dietary salt beyond blood pressure. CurrHypertens Re,2019;21(6):42-52.
5. Cappuccio FP. Cardiovascular and other effects of salt consumption. Kidney IntSuppl, 2013;3(4):312–315.
6. Sanders PW. Vascular consequences of dietary salt intake. Am J Physiol Renal Physiol, 2009; 297(2): F237–43.
7. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. Cochrane Database Syst Rev, 2011;11:CD004022.
8. Campino C, Baudrand R, Valdivia CA, Carvajal C, Vecchiola A, Tapia-Castillo A et al. Sodium intake is associated with endothelial damage biomarkers and metabolic dysregulation. Am J Hypertens, 2018; 31(10): 1127–1132.
9. Choi JW, Park JS, Lee CH. Interactive effect of high sodium intake with increased serum triglycerides on hypertension. PLoS One, 2020;15(4): e0231707.
10. Hou L, Zhang M, Han W, Tang Y, Xue F, Liang S *et al*. Influence of salt intake on the association of blood uric acid with hypertension and related cardiovascular risk. PLoS One1, 2016;1(4):e0150451.
11. Wang Y, Chu C, Wang KK, Hu JW, Yan Y, LvYB *et al*. Effect of salt intake on plasma and urinary uric acid levels in Chinese adults: An interventional trial. Sci Rep, 2018; 8(1):1434.
12. Choi YJ, Yoon Y, Lee KY, Hien TT, Kang KW, Kim KC *et al*. Uric acid induces endothelial dysfunction by vascular insulin resistance associated with the impairment of nitric oxide synthesis. FASEB J, 2014;28(7):3197–3204.
13. Albu A, Para I, Porojan M. Uric acid and arterial stiffness. TherClin Risk Manag, 2020;16:39-54.
14. Sica DA. Pharmacokinetics and pharmacodynamics of mineralocorticoid blocking agents and their effects on potassium homeostasis. Heart Fail Rev, 2005;10(1): 23–29.
15. Alderman MH Reducing dietary sodium: the case for caution. JAMA, 2010;303(5):448- 449.
16. Graudal NA, Hubeck-Graudal T, Jürgens G. Reduced dietary sodium intake increases heart rate. A meta-analysis of 63 randomized controlled trials including 72 study populations Front Physiol, 2016;7:111.
17. Tokoudagba J, Auger C, Bréant L, N'Gom S, Chabert P, Idris-Khodja N *et al*. Procyanidin-rich fractions from *Parkia biglobosa* (Mimosaceae) leaves cause redox-sensitive endothelium-dependent relaxation involving NO and EDHF in porcine coronary artery. J Ethnopharmacol, 2010;132(1):246-250.
18. Gbolade A. Ethnobotanical study of plants used in treating hypertension in Edo State of Nigeria. J Ethnopharmacol, 2012;144(1):1-10.
19. Tijani AY, Okhale SE, Salawu TA, Onigbanjo HO, Obianodo LA, Akingbasote JA *et al*. Antidiarrhoeal and Antibacterial properties of crude aqueous stem

bark extract and fractions of *Parkia biglobosa* (Jacq.) R. Br. Ex G. Don. Afr J Pharm Pharmacol, 2009;3: 347–353.
20. Sofola OA, Knill A, Hainsworth R, Drinkhill M Change in endothelial function in mesenteric of arteries of Sprague-Dawley rats fed a high salt diet. J Physiol,2002;543(Pt 1):255–260
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. ClinChem, 1972;18(6): 499–502.
22. Kassi Y, Aka KJ, Abo KJ, Mea A, Bi SAN, Ehile EE. Effetantihypertensif d' un extraitaqueux d' écorce de tronc de *Parkia biglobosa* (mimosaceae) sur la pressionartérielle de lapin. Sci Nat, 2008;5(2): 133-143.
23. Ouédraogoa S, Somé N, Ouattara S, Kini FB, Traore A, Bucher B et al. Acute toxicity and vascular properties of seed of *Parkia biglobosa* (JACQ) R. Br Gift (Mimosaceae) on rat aorta. Afr J Tradit Complementary Altern Med, 2012;9(2):260–265
24. Kang YJ, Wang HW, Cheon SY, Lee HJ, Hwang KM, Yoon HS. Associations of obesity and dyslipidemia with intake of sodium, fat, and sugar among Koreans: a qualitative systematic review. ClinNutr Res, 2016;5(4):290–304.
25. Odetola AA, Akinloye O, Egunjobi C, Adekunle WA, Ayoola AO. Possible antidiabetic and antihyperlipidaemic effect of fermented *Parkiabiglobosa* (jacq) extract in alloxan induced diabetic rats. ClinExpPharmacolPhysiol, 2006;33(9):808-812.
26. Zou B, Li C, Chen J, Dong X, Zhang Y, Du J. High molecular weight persimmon tannin is a potent hypolipidemic in high-cholesterol diet fed rats. Food Res Int, 2012;48(2):970-977.
27. Fan H, Wu D, Tian W, Ma X. Inhibitory effects of tannic acid on fatty acid synthase and 3T3-L1 pre-adipocyte. BiochimBiophysActa, 2013; 1831(7): 1260-1266.
28. Xu H, Yu X, Qu S, Chen Y, Wang Z, Sui D. *In vivo* and *in vitro* cardioprotective effects of Panax quinquefolium 20 (S)-protopanax adiolsaponins (PQDS), isolated from Panax quinquefolium. Pharmazie, 2013;68:287-292.
29. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K *et al*. High- sensitivity C-reactive protein and cardiovascular disease. A resolute belief or an elusive link? J Am CollCardiol, 2013;62(5):397–408.
30. Kouadio F, Kanko C, Juge M, Grimaud N, Jean A, N'guessan Y *et al*. Analgesic and antiinflammatory activities of an extract from *Parkia biglobosa* used in traditional medicine in the Ivory Coast. Phytother Res, 2000;14:635-637.
31. Nwaehujor CO, Ezeigbo I, Udeh NE, Ezeja MI, Asuzu IU. Anti-inflammatory and anti-oxidant activities of the methanolic extracts of the stalk of *Parkia biglobosa* (JACQ.) BENTH. Hygeia J D Med, 2011;3(1):34-40.
32. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res, 2017;120(4):713-735.
33. Boegehold MA. The effect of high salt intake on endothelial function: reduced vascular nitric oxide in the absence of hypertension. J Vasc Res, 2013;50(6): 458-467
34. Lee SJ, Oh BK, Sung KC. Uric acid and cardiometabolic diseases. Clin Hypertens, 2020;26: 13.
35. Kushiyama A, Nakatsu Y, Matsunaga Y, Yamamotoya T, Mori K, Ueda K *et al*. Role of uric acid metabolism-related inflammation in the pathogenesis of metabolic syndrome components such as atherosclerosis and nonalcoholic steatohepatitis. Mediators Inflamm, 2016;2016:8603164. doi:10.1155/2016/8603164
36. Sharaf El Din UAA, Salem MM, Abdulazim DO. Uric acid in the pathogenesis of metabolic, renal, and cardiovascular diseases: A review. J Adv Res, 2017;8(5):537-548