



Research Article

Effects of *Psidium guajava* and *Ribes americanum*
Aqueous Extracts and Dietary Modification on some
Markers of Renal, Cardiac Dysfunction in Rats

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ARTICLE INFO:

ABSTRACT

Keyword:

Psidium guajava
Ribes americanum
Renal function
Cardiac function

The effect of aqueous extract of *P. guajava* and *R. americanum* and dietary modification interactions on serum renal function markers levels (urea, creatinine) cardiac function markers (lactate dehydrogenase, creatinine kinase), serum ions (calcium, sodium, and potassium ions) were investigated in rats. Fully grown Wister strain rats were exposed to high fat and salted diet formulation and treated with the aqueous extract for a period of 6 weeks. The diets were formulated by adding 25% animal fat and 5% palm oil to standard feeds (Vital feeds, Bukuru Jos, Plateau. Significant decrease in serum urea, creatinine, and lactate dehydrogenase and creatine kinase activities in the group treated with *P. guajava* and *R. americanum* aqueous extract compared to the high fat-salted water group. There was no significant difference in plasma urea and Creatinine, LDH and CK levels between the standard feeds and standard feed plus salted water group. Significant increase in the plasma Creatinine and lactate dehydrogenase levels (at $p < 0.05$) of the high-fat plus salted water group when compared with the high fat group. Significant (at $p < 0.05$) increase in serum sodium of the group exposed to salted water when compared with the non-salted water group. There was a significant ($p < 0.05$) decrease in serum sodium levels of extract administered groups compared to the high fat-salted water and standard feed salted water groups. There was also a significant increase, at $p < 0.05$, in serum potassium and calcium of the extracts administered group when compared with the standard feed salted water, high fat and high fat salted water groups. The study showed that the combined effects of high fat and high salt increased serum sodium and decreased serum potassium and calcium. Administration of the aqueous extract administration reversed by the effects.

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INTRODUCTION

Herbal remedies, comprise therapies based on the holistic treatment of physical disorders, generally addressing the causes of diseases rather than their symptoms and taking steps in the prevention of disease. It is for this reason that the National Centre for Complementary and Alternative Medicine was established by the United States Government to study the development of herbal medicine (Mallick *et al.*, 2006).

Sodium has been of interest in public health nutrition for decades, mainly because of it being associated with hypertension and CVDs, especially coronary heart disease (CHD) and stroke. Additionally, diets that are high in sodium may have independent but additive harmful effects on left ventricular hypertrophy, progression of renal disease, and risk of CVD and stroke. High sodium intake also presents a challenge for excretion by the kidneys, which is another potential mechanism for affecting blood pressure and the risk of diseases. Increased sodium intake may lead to increased urinary protein excretion and may thus increase the rate of deterioration of renal function (WHO, 2003; He and MacGregor, 2009). Studies have demonstrated a decrease in urinary protein excretion and a significant decrease in protein: creatinine ratio with a decrease in salt consumption (Vogt *et al.*, 2008). Likewise, a decrease in urinary protein excretion with reduced sodium intake relative to control was statistically significant (Swift *et al.*, 2005). The Suckling study also reported a significant reduction in albumin: creatinine ratio ($P= 0.014$) for the reduced sodium group relative to the control (WHO, 2010; Melander *et al.*, 2007; He *et al.*, 2009). *Psidium guajava* leaves have been used to treat cough and pulmonary diseases in Bolivia and Egypt; they have also been used to treat cough in India and as an anti-inflammatory and hemostatic agent in China. The plant has

been used in Africa and Asia to prevent and treat scurvy and to treat hypertension in western Africa. Ethnomedicinal reports document the use of the plant in treating malaria. Scientific investigations of the medicinal properties of guava leaf products date back to the 1940s (USDA, 2011). *Ribes americanum* (Blackcurrent) plant belongs to the species of Ribes berry native found commonly in central, northern Europe and Northern Asia. Black currant was used for centuries in Chinese folk medicine as a diuretic, diaphoretic and febrifuge, and as an ingredient in nutraceuticals, wines, juices and jams in China and Europe (Suzutani *et al.*, 2003, Matsumoto *et al.*, 2005; Del Castillo *et al.*, 2004).

In an animal model, a water-alcohol extract of *P. guajava* depressed guinea pig arterial contractility in a concentration-dependent manner. In hypertensive rats, intravenous administration of guava leaf aqueous extracts produced a dose-dependent reduction in systemic arterial blood pressure and heart rate (Ojewole, 2005). Several studies have focused on the therapeutic potential of blackcurrant regarding hypertension and other cardiovascular-associated illnesses, neoplastic, neurodegenerative and ocular diseases and diabetic neuropathy (Gopalan *et al.*, 2012).

This study was aimed at investigating the combined effects of high-fat diet, high salt intake and the treatment with *P. guajava* and *R. americanum* on some serum renal and cardiac markers to establish the likely harmful effects of high fat and salt and the benefits of the extracts in preventing and treating the negative effects on renal and cardiac function.

MATERIALS AND METHODS

The Plants and Extraction Procedure:

Ribes americanum and *Psidium guajava* leaves were harvested from plants in Bayero University campus, Kano and authenticated by a taxonomist

from the Faculty of Sciences, Bayero University. The leaves were washed, dried in a fully ventilated environment and away from direct sun light. The dried leaves were ground using a mechanical grinder.

Leaves aqueous extract of *Ribes americanum* and *Psidium guajava* were prepared according to Mittal *et al.* (1981) and Fernando *et al.* (1989) methods. Two hundred grams (200g) of powdered leaves of *Ribes americanum* or *Psidium guajava* was mixed with one litre of distilled water in a 5-litre beaker and steamed at 65^oc for one hour then allowed to cool and mixed vigorously. The mixture was filtered using Whatman No. 1 filter paper. The aqueous extract was then concentrated by evaporation at 60^oc in water bath (OLS 200 water bath and shaker, Grand instruments, Cambridge). Finally, the total yield of the aqueous extract was determined and the extract stored in air tied containers.

Experimental animals and Grouping

Fully grown Wister strain rats were exposed to high fat diet formulation for a period of 6 weeks. The diet was formulated by adding 25% animal fat and 5% palm oil to standard feeds (vital feeds, Bukuru jos, Plateu) and body weights (BW) were recorded weekly (Boustany-Kari *et al.*, 2007)

GROUP I: standard feed (Broilers finisher) + non salted water

GROUP II: standard feed (Broilers finisher) + salted water

GROUP III: high fat diet + non salted water

GROUP IV: High fat diet + salted water

GROUP V: high fat diet + Salted water + 350 mg/ kg *Psidium guajava* aqueous extract

GROUP VI: high fat diet + Salted water + 350 mg/ kg *Ribes americanum* aqueous extract

At the end of the experimentation, the rats were sacrificed by decapitation and blood was collected for further studies.

Effects of the Treatment and administration on Renal Function and Cardiac Enzymes:

Determination of blood urea:

Blood urea was determined using diacetyl method of Natelson (1951), using Randox

laboratory kits and procedure. When urea is strongly heated in acidic conditions in the presence of substances containing two adjacent carbonyl groups a yellow compound is formed which is converted to a red complex by ferric ions. The intensity of the red complex is proportional to the concentration of the urea.

Determination of serum Creatinine:

Serum creatinine was determined using Randox laboratory test kits and procedure. Red colour is produced by creatinine with alkaline solution of picric acid (Jaffes reaction). The red colour is photometrically measured at 520nm.

Lactate dehydrogenase assay:

Lactate dehydrogenase was assayed using Randox laboratory test kits and procedure. Lactate dehydrogenase (LDH) catalyses the reduction of pyruvate to lactate in the presence of NADH⁺

The initial rate of NADH oxidation is directly proportional to the catalytic LDH activity. It is determined by measuring the decrease in absorbance at 340 nm

Creatine kinase assay

Creatine kinase (CK) was assayed using Randox laboratory test kits and procedure. Creatine kinase (CK) catalyses the phosphorylation of ADP, in the presence of Creatine phosphate, to form ATP and creatinine. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm by means of hexokinase and glucose-6-phosphate dehydrogenase couple reaction.

Determination of Serum Na, K and Ca Ions **Determination of serum Na and K by Flame photometry**

The concentrations of Sodium and potassium ions were determined using flame emission photometry method of Magoshes and Vallee (1956).

Colometry determination of serum Ca ion

Serum calcium ion concentration is determined by the chromogenic complex formed between calcium ions and o-

cresolphthalein, which is measured at 575 nm and is proportional to the concentration of calcium ions present.

Statistical analysis

The results of the studies were presented as MEANS ± SD and the difference between the means was analysed using statistical software, Grapad-instant, version 3.0.

RESULTS

Results for the effects of dietary modification and *Psidium guajava* and *Ribes americanum* leaves aqueous extract on urea, creatinine, lactate dehydrogenase and creatine kinase

There was no significant difference in plasma urea and Creatinine, lactate dehydrogenase and creatine kinase levels between the standard feeds and standard feeds-salted water groups. Likewise, there was no significant

difference in plasma urea levels and creatine kinase activity of the high-fat and high-fat-salted water groups. However, there was a significant (at p<0.05) increase in the plasma Creatinine and lactate dehydrogenase activity of the high-fat-salted water group when compared with the high fat group.

The result gave a significant decrease in serum urea and Creatinine activity of the groups treated with *P. guajava* and *R. americanum* aqueous extract when compared with the high fat-salted water group. Likewise, there was a significant decrease in lactate dehydrogenase and creatine kinase activity of the group treated with *P. guajava* and *R. americanum* aqueous extract when compared with the high fat-salted water group.

Table 1: Result of 6 weeks dietary modification and *Psidium guajava* and *Ribes americanum* leaves aqueous extract on urea, creatinine and lactate dehydrogenase and creatine kinase of rats

n=5	Urea (µmol/L)	Creatinine (µmol/L)	Lactate dehydrngenase(IU/l)	Creatine Kinase(IU/l)
Standard feed only	5.10±0.82 ^a	72.02±1.03 ^a	203.25±12.12 ^a	72.22±5.21 ^a
Standard feeds + high salt	5.61± 0.90 ^a	82.43±3.23 ^{bd}	213.98±9.21 ^{ab}	78.05±6.42 ^a
High fat only	5.98±1.12 ^{ab}	84.67±4.12 ^b	221.22±6.74 ^b	86.28±4.33 ^b
High fat + high salt	6.56±1.20 ^b	95.93±2.65 ^c	231.06±5.28 ^c	91.20±6.12 ^b
Highfat +salt + 350 mg/kg <i>p. guajava</i>	5.19±1.02 ^a	78.58±6.01 ^d	218.25±2.85 ^b	77.28±8.15 ^a
High fat+salt + 350mg/kg <i>R. americanum</i>	5.12±0.87 ^a	81.22±5.33 ^{bd}	208.22±8.10 ^a	74.20±7.01 ^a

Result presented as mean ± SD, Values with different superscript along a column are statistically significant

The effects of Dietary modification

The result indicated a significant increase in the serum sodium concentration of the group exposed to salted water when compared with the non-salted water group. There was no significant

(p< 0.05) difference in the serum potassium and calcium levels of the high-fat salted-water group, high-fat group, and standard feed salted-water groups. However, there is a significant (at p< 0.05) decrease in serum potassium and calcium

of the groups when compared with standard feeds. Significant (at $p < 0.05$) decrease in serum sodium levels of *P. guajava* and *R. americanum* leaves extract administered groups when compared with high fat salted water group, and standard feed salted water group was

observed. Likewise, a significant ($p < 0.05$) increase in serum potassium and calcium of the extracts administered group when compared with the standard feed-salted water, high fat feeds, and high fat-salted water groups were recorded.

Table 2: Results of *P. guajava* and *R. americanum* leaves aqueous extract on serum Na, K and Ca levels in rats fed high fat and salted water.

n=5	Na(meq/L)	K(meq/L)	Ca(mg/dl)
Standard feeds	134.56 ± 5.22 ^a	8.56 ± 1.02 ^{ab}	9.50 ± 1.22 ^{ac}
Standard feeds + salt	185.20 ± 8.31 ^b	7.50 ± 2.11 ^a	8.15 ± 0.95 ^b
High fat	145.80 ± 12.01 ^c	8.22 ± 1.08 ^a	8.35 ± 1.05 ^{ab}
High fat feeds + salt	219.25 ± 16.21 ^d	8.05 ± 2.05 ^a	7.68 ± 0.85 ^b
Standard feeds + 350mg/kg <i>P. guajava</i>	160.50 ± 9.50 ^{eg}	9.65 ± 1.57 ^b	9.84 ± 1.11 ^c
Standard feeds + + 350mg/k <i>R. americanum</i>	155.4 ± 11.10 ^{ce}	11.56 ± 2.01 ^c	10.25 ± 0.98 ^c
High fat feeds + salt +350mg/kg <i>P. guajava</i>	176.54 ± 15.00 ^f	9.75 ± 1.25 ^b	9.64 ± 1.01 ^c
High fat feeds + salt + 350mg/kg <i>R. americanum</i>	168.40 ± 9.45 ^{fg}	10.98 ± 2.40 ^{bc}	10.55 ± 1.02 ^c

Result shown as mean ± SD, different superscript along a column are statistically significant

DISCUSSION

The result for the effect of *P. guajava* and *R. americanum* leaves aqueous extract on serum levels of urea and creatinine and the serum activities of lactate dehydrogenase and creatine kinase (Table 2) indicated significant ($p < 0.05$) decrease in the amount of serum urea and creatinine of the groups treated with *P. guajava* and *R. americanum* aqueous extract as compared with the high fat-salted water group. Likewise, there was significant decrease in lactate dehydrogenase and creatine kinase activities of the group treated with *P. guajava* and *R. americanum* aqueous extract as compared with the high fat-salted water group. However, significant ($P < 0.05$) difference in plasma urea and creatinine levels and the activities of LDH and CK between the standard feed and standard feed-salted water groups was not observed. Likewise, no significant ($p < 0.05$) difference in serum urea level and creatine kinase activity of the high fat and high fat-salted water groups was

observed. However, significant ($p < 0.05$) difference in the serum creatinine level and lactate dehydrogenase activity of the high fat and high fat-salted water groups. This study agreed with, Swift (2005), Vogt *et al.* (2008) and Fotherby *et al.* (1993) who reported that salt consumption compromises renal function without necessarily affecting the structural integrity of the kidney (WHO, 2012).

This study showed that individual effects of high fat or high salt did not significantly affect renal and cardiac markers, but the combine effects of high fat and high salt significantly affects the levels of the markers. The study also indicated that, *P. guajava* and *R. americanum* extracts administration significantly affect the negative effects of high fat and salted water by the reducing the level of the renal and cardiac markers to normal levels.

The effects of *P. guajava* and *R. americanum* aqueous extract on serum sodium, potassium and calcium levels of rats fed on high fat and salted

water (Table 2) showed that significant ($p < 0.05$) increase in serum sodium of the group exposed to salted water when compared with the non-salted water group was observed. However, no significant ($p < 0.05$) difference in the serum potassium and calcium levels of the high fat feed salted water, high fat feeds and standard feed-salted water groups was observed. There was also significant ($p < 0.05$) decrease in serum sodium levels of *P. guajava* and *R. americanum* leaves extract administered groups when compared with the high fat-salted water and standard feed salted water groups. Likewise, there was significant ($p < 0.05$) increase in serum potassium and calcium of the extracts administered group when compared with the standard feed salted water, high fat, and high fat salted water groups. The study showed that the combine effects of high fat and high salt increased serum sodium and decreased serum potassium and calcium. However, the effect was reversed by the extract administration.

This finding was supported by previous studies which reported increase in serum sodium and decrease in serum potassium and calcium of rats exposed to high fat and salt. Kang *et al.* (2002) reported that hypernatremia is rare but does occur when there is loss of body fluids containing less sodium than plasma along with water intake restriction or if there is excessive sodium intake with limited liquid intake. Wilde *et al.* (2000) observed that obesity may increase vascular responsiveness by elevated extracellular calcium influx through L-type calcium channels.

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CONCLUSION

This study showed that individual effects of high fat or high salt did not significantly affect renal and cardiac markers, but the combine effects of high fat and high salt significantly affects the levels of the markers. The study also indicated that, *P. guajava* and *R. americanum* extracts administration significantly affect the negative effects of high fat and salted water by the reducing the level of the renal and cardiac markers to normal levels.

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ACKNOWLEDGMENT

We will acknowledge the support of Chemistry Department, Bayero University, Kano for support with reagents and the Departmental AAS machine for the study. The Department of Biochemistry also made the research laboratory equipment available for our studies.

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