Journal of Research in Applied and Basic Medical Sciences 2024; 10(4): 347-360

Original Article

RABMS Journal of Research in Applied and Basic Medical Sciences

Ameliorative action of *Tetrapleura tetraptera* fruit extract on the kidney of Swiss mice following chronic dietary salt intake

Florence M. Ekong[1], Akpan U. Ekanem[1], Aquaisua N. Aquaisua[1], Moses B. Ekong*[1]

[1]*Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria*

***Corresponding author:** Moses B. Ekong, **Address**: Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria, **Email**: mosesekong@uniuyo.edu.ng, **Tel**: +234(0)-8030868505

Abstract

***Background & Aims*:** Dietary salt is a common compound used for both food and non-food purposes. It is beneficial to the bodies of animals due to its electrolyte composition, which is necessary for several metabolic processes. However, higher quantities in the body can have deleterious effects on vital tissues. To manage such adverse effects on the kidney, the present study investigated the role of an antioxidant-rich plant, *Tetrapleura tetraptera*, on kidney function and structure in Swiss mice.

***Materials & Methods*:** Twenty-five Swiss mice were equally divided into groups: control, dietary salt only, and dietary salt with interventions of *T. tetraptera* at 250 mg/kg and 500 mg/kg, and Losartan. The administrations were by oral gavage, and lasted for eight weeks. Upon sacrifice, serum creatinine, electrolytes, kidney histology, and collagen were studied.

***Results*:** The Swiss mice gained weight in the dietary salt group, but the kidney somatic indices and serum creatinine levels were not affected. However, serum sodium ion levels were significantly ($p < 0.05$) higher, while there was no difference in serum chloride, potassium, calcium, phosphorus, and magnesium ions. Kidney histology showed hypertrophied glomeruli with reduced Bowman's capsular spaces and less collagen distribution. The *T. tetraptera* groups at 250 mg/kg and 500 mg/kg showed improved renal function with reduced sodium levels, improved glomerular appearance, and collagen distribution compared to the control.

***Conclusion*:** *T. tetraptera* at 250 mg/kg and 500 mg/kg demonstrated ameliorative or protective effects against the adverse impacts of dietary salt intake.

***Keywords*:** Collagen, Creatinine, Electrolytes, Glomerulus, Sodium chloride, *Tetrapleura tetraptera*

Received 13 September 2023; accepted for publication 14 August 2024

Introduction

Dietary salt, more scientifically called sodium chloride, is an essential ingredient for humankind. It is used in almost every food or food item to enhance flavour (1,2). Industrially, it is a vital component of many manufactured compounds, including medicine, household items, and other industrial goods. It is mostly sourced from seawater and also from mining rock deposits (2). Dietary salt provides sodium and chloride ions, which are essential for most of the body's metabolic processes (3).

The retention of dietary salt in the body depends on the amount of intake and its elimination. In societies with a greater prevalence of processed foods, dietary

salt intake is usually high, with an equally high rate of elimination (4,5). However, the World Health Organization recommends a dietary salt intake of less than 5 g per day to avoid its deleterious effects, including cardiovascular disease and its associated costs (6). Excess dietary salt electrolytes are easily excreted through urine and sweat (4,7,8). A high-salt diet increases serum sodium chloride levels, leading to water retention, increased blood volume, and pressure, which strain the kidneys, leading to diseases and failure (9,10). Furthermore, if the excess of these electrolytes cannot be eliminated, they build up in body tissues, a condition known as hypernatremia and hyperchloremia (11–15). A build-up of such electrolytes in the kidneys triggers other metabolic activities, leading to serious consequences, such as fluid retention, which increases blood volume and blood pressure.

High dietary salt intake is also implicated in the onset of high blood pressure in normotensive individuals and can further exacerbate the condition in salt-sensitive individuals and experimental animals (16–19). Salt-induced high blood pressure and hypertension affect kidney function and structure and cause ventricular atrophy and damage to blood vessels (16,20,21).

The kidneys balance the levels of electrolytes and waste products by conserving or releasing water. High salt intake is reported to induce glomerular damage (22,23), which may decrease effective renal blood flow and concomitantly increase the filtration fraction and glomerular capillary pressure (16). The uniqueness of the kidneys makes them a target for studying hypertension and screening potential antihypertensive agents. Since much of the excess dietary salt, a predisposing factor for high blood pressure, is consumed in food, how can this be modulated to protect the kidneys and other body tissues from damage?

Natural remedies in plants have been reported to modulate blood pressure. Because of the abundance of active components within these plants, it is suggested that they may control blood pressure through more than a single mechanism. *Tetrapleura tetraptera* (*T. tetraptera*) has been reported to have blood pressure-reducing potential and other medicinal importance in experimental animals (24–26). Can the blood pressure reduction by *T. tetraptera* protect the kidneys? The plant *T. tetraptera* belongs to the Fabaceae family. In Nigeria, it is mainly used as a spice, medicine, and dietary supplement (27). It is possible that *T. tetraptera* acts by reducing sodium levels, thereby restoring kidney function and, eventually, blood pressure. This study evaluated the potential protective effects of *T. tetraptera* fruit extract on kidney function in Swiss mice subjected to chronic high-salt diets.

.

Materials & Methods

Animal Handling

A total of 25 male Swiss mice were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The Swiss mice were kept in clean cages and acclimatized for a period of 14 days under a natural 12 hr light/dark cycle and a temperature of 26-29 ℃. The Swiss mice were allowed standard chow and water *ad libitum*. Ethical approval was obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee with Number: UU_FBMSREC_2022_002. All the Swiss mice were handled following the guidelines for the use of animals in laboratory research (28). To minimize stress, no animal was handled for more than 30 seconds at a time, and they were not exposed to any painful stimuli.

Plant Sourcing and Preparation

Mature fruits of *Tetrapleura tetraptera* were obtained from a local farm in Adim, Biase Local Government Area of Nigeria. The plant was identified and authenticated in the Department of Botany at the University of Uyo, and the voucher number UUPH/A32(f) was deposited at the herbarium unit. The cleaned fruits were air-dried and pulverized with an electric blender. The powdered *Tetrapleura tetraptera* fruit was extracted in 80% ethanol for 72 hr, filtered, and evaporated to dryness in a water bath. The dry extract was preserved at 4 ℃. At the same time, the

phytochemicals of *T. tetraptera* were screened using standard techniques to detect the various constituents.

Exactly 2 grams of the *T. tetraptera* extract was weighed and dissolved in distilled water each day to be administered to the animals orally at 250 mg/kg and 500 mg/kg body weight.

Procurement of Sodium Chloride

Sodium chloride was obtained as dietary salt from a local market in Uyo Metropolis, Nigeria, and stored in an airtight container at room temperature to protect it from moisture. Distilled water was used as the vehicle to dissolve the salt. A fresh solution of 9% volume/volume dietary salt was prepared daily.

Experimental Design

The 25 Swiss mice were grouped into five groups: Group 1 was the control (administered 10 ml/kg of distilled water), and Group 2 was administered dietary salt in distilled water (90 mg/ml, (24). Groups 3-5 were administered dietary salt (90 mg/ml), along with interventions of *T. tetraptera* (250 mg/kg), *T. tetraptera* [500 mg/kg, (29,30)], and Losartan (50 mg per day), respectively. All administrations were by oral gavage and lasted for 8 weeks (4 weeks of dietary salt only and 4 weeks of intervention concomitantly with the dietary salt). The body weights were measured weekly.

Termination of the Experiment

The Swiss mice were anesthetized with 50 mg/kg body weight ketamine hydrochloride (i.p.) after overnight fasting and were then sacrificed. Blood was obtained through intra-cardiac puncture for serum creatinine and electrolyte analyses. The kidneys were collected through an incision in the thoraco-abdominal wall, weighed, and fixed in 10% buffered formalin. The kidneys were subsequently routinely processed for paraffin wax embedding, sectioned at 8 µm thickness, and further processed for histology using the hematoxylin and eosin technique, and histochemistry using the Masson's trichrome technique (31).

The blood samples were centrifuged at 1,500 rpm to obtain the sera. The sera were stored in plain tubes for further analyses of creatinine and electrolyte levels. Serum creatinine measurement was performed by an isotope dilution mass spectrometry-traceable enzymatic method using a Roche Modular Diagnostic GmbH with intra- and inter-assay coefficients of variation of 0.9% and 2.9%, respectively. The serum electrolyte levels were determined by the ion-selective electrode principle of the COBAS 6000 (c501) analyzer (32). The following electrolytes were assayed: sodium, potassium, chloride, calcium, phosphorus, and magnesium ions.

Statistical Analysis

All the data were analyzed using GraphPad Prism (version 5.0), and the results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance was used to compare the data of the control and test groups, while the post hoc Tukey's multiple comparison test was applied to determine the differences among groups. Results with probability levels at $p \leq 0.05$ were regarded as significant.

Results

Phytochemical Screening

The phytochemical screening of *T. tetraptera* fruit was conducted to identify the most active medicinal constituents. The qualitative screening of *T. tetraptera* showed the presence of alkaloids, flavonoids, saponins, glycosides, polyphenols, terpenoids, tannins, terpenes, and steroids.

Body Weight Change

During the course of the experiment, there was body weight gain in all the experimental groups, with higher gains observed in the control and the group administered dietary salt with the intervention of 250 mg/kg *T. tetraptera* (Table 1).

Table 1. Body weight change of the experimental groups

Group (n = 5)	Initial body weight (g)	Final body weight (g)	Body weight change (g)	Body weight change (%)
Control	25.60 ± 1.12	28.00 ± 0.55	2.40	8.57
Dietary salt (90 mg/mL)	27.80 ± 0.73[a]	28.20 ± 0.66[a]	0.40	1.42
Dietary salt (90 mg/mL) and *T. tetraptera* (250 mg/kg)	25.20 ± 0.49[a]	28.40 ± 0.87[a]	3.20	11.27
Dietary salt (90 mg/mL) and *T. tetraptera* (500 mg/kg)	29.00 ± 1.30[a,b]	30.20 ± 0.97[a]	1.20	3.97
Dietary salt (90 mg/mL) and Losartan	31.80 ± 0.49***	32.80 ± 0.49**	1.00	3.05

ANOVA and Tukey post hoc test

Data are presented as Mean ± Standard error of Mean

** - Significantly different from the control at $p < 0.05$

*** - Significantly different from the control at $p < 0.05$

a - Significantly different from the Losartan group at $p < 0.05$

b - Significantly different from the dietary salt group at $p < 0.05$

Organ Weight and Organo-Somatic Indices

The kidney weights were not significantly ($p > 0.05$) different between the test groups administered dietary salt only or with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera*, and Losartan compared to the control. The organo-somatic indices were similar among the experimental groups (Table 2).

Table 2. Organ weights and organo-somatic indices

Group (n = 5)	Kidney weight (g) $P = 0.1595$, F = 1.846	Kidney-somatic index
Control	0.30 ± 0.01	1.07
Dietary salt (90 mg/mL)	0.34 ± 0.01	1.21
Dietary salt (90 mg/mL) and *T. tetraptera* (250 mg/kg)	0.30 ± 0.02	1.06
Dietary salt (90 mg/mL) and *T. tetraptera* (500 mg/kg)	0.31 ± 0.02	1.03
Dietary salt (90 mg/mL) and Losartan	0.35 ± 0.02	1.07

ANOVA and Tukey post hoc test

Data are presented as Mean ± Standard error of Mean

Not significantly different from the control at $p < 0.05$

Serum Creatinine

The serum creatinine levels were not significantly ($p = 0.0394$) different between the test groups (administered dietary salt only or with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera*, and Losartan) and the control. However, there was a significantly higher ($p < 0.05$) serum creatinine level in the dietary salt compared to the Losartan group (Table 3).

Table 3. Serum creatinine levels of the experimental groups at 8 weeks

Group (n = 5)	**Creatinine level (mg/dL)** $P = 0.0394$; F = 3.084
Control	0.20 ± 0.03
Dietary salt (90 mg/mL)	0.27 ± 0.05
Dietary salt (90 mg/mL) and *T. tetraptera* (250 mg/kg)	0.24 ± 0.02
Dietary salt (90 mg/mL) and *T. tetraptera* (500 mg/kg)	0.17 ± 0.01
Dietary salt (90 mg/mL) and Losartan	0.16 ± 0.01[a]

ANOVA and Tukey post hoc test

Data are presented as Mean ± Standard error of Mean

a - Significantly different from the Dietary-salt group at $p < 0.05$

Serum Electrolytes

The serum sodium ion was significantly (p = 0.0001) higher in the test groups administered dietary salt only or with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera*, and Losartan compared to the control. However, there was no difference in serum sodium ion between the test groups administered dietary salt only or with the intervention of 500 mg/kg *T. tetraptera* compared to the control (Table 4).

The serum chloride ion was significantly lower (p=0.019) in the test group administered dietary salt with the intervention of Losartan compared to the control. However, there was no difference in serum chloride ion between the test groups administered dietary salt only or with interventions of 250 mg/kg and 500 mg/kg *T. tetraptera* compared to the control (Table 4)

There was no difference in serum potassium (p = 0.101), calcium (p = 0.632), phosphorus (p = 0.072), and magnesium ($p < 0.05$) ions between the test groups and the control. However, the serum magnesium ion was significantly (p = 0.004) lower in the group administered dietary salt only compared to the groups administered dietary salt with interventions of 250 mg/kg and 500 mg/kg *T. tetraptera* (Table 4).

Table 4. Serum ion levels of the experimental groups

Group (n = 5)	**Sodium** (mmol/L) P =0.0001 F = 9.813	**Potassium** (mmol/L) P = 0.101 F = 2.240	**Chloride** (mEq/L) P = 0.019 F = 3.794	**Calcium** (mg/dL) P = 0.632 F = 0.653	**Phosphorus** (mg/dL) P = 0.072 F = 2.541	**Magnesium** (mg/dL) P = 0.004 F = 5.455
Control	117.50± 0.63	8.45 ± 0.23	149.90 ± 4.99	10.38 ± 0.36	4.07 ± 0.20	2.00± 0.07
Dietary salt-only	121.50 ± 0.36**	8.69± 0.09	139.40 ± 1.22	9.88± 0.62	3.66 ± 0.07	1.84± 0.05
Dietary salt and *T. tetraptera* (250 mg/kg)	122.40 ± 0.32***	8.86 ± 0.13	143.50± 1.41[b]	10.64 ± 0.13	3.47 ± 0.12	2.15± 0.04[a]
Dietary salt and *T. tetraptera* (500 mg/kg)	121.10 ± 0.58**	9.06 ± 0.16	145.70 ± 0.96[b]	10.33 ± 0.33	3.74 ± 0.25	2.09± 0.06[a]

Group (n = 5)	Sodium (mmol/L) P =0.0001 F = 9.813	Potassium (mmol/L) P = 0.101 F = 2.240	Chloride (mEq/L) P = 0.019 F = 3.794	Calcium (mg/dL) P = 0.632 F = 0.653	Phosphorus (mg/dL) P = 0.072 F = 2.541	Magnesium (mg/dL) P = 0.004 F = 5.455
Dietary salt and Losartan	121.40 ± 0.94**	8.91 ± 0.13	130.10 ± 1.99***	10.56 ± 0.20	3.39 ± 0.12	1.92± 0.03[b]

ANOVA and Tukey post hoc test

Data are presented as Mean ± Standard error of Mean

** - Significantly different from the control at $p < 0.05$, *** - Significantly different from the control at $p < 0.05$

a-Significantly different from the dietary salt group at $p < 0.05$, b-Significantly different from the Losartan group at $p<0.05$

Histology of the Kidney

The kidney histology consists of an outer cortex and an inner medulla. The cortex shows nephrons consisting of renal corpuscles. Each renal corpuscle is composed of glomeruli surrounded by a double-walled epithelial Bowman's capsule, which is continuous with the proximal convoluted tubule at the tubular pole. Each proximal convoluted tubule is lined by a single layer of columnar cells. Also present are the distal convoluted tubules, which are lined by a single layer of cuboidal cells. The outer parietal layer of Bowman's capsule is lined by a single squamous epithelium on a basement membrane, while the visceral layer of this capsule closely envelops the glomerular capillaries. In the section of the control group, all cell types appear normal, with prominent parietal capsular cells (Figure 1). The kidney histology of the test groups showed similar cortical and medullary arrangements to the control. However, Group 2, administered dietary salt alone, showed hypertrophied glomeruli with a reduced Bowman's capsular space and less prominent parietal capsular cells. The proximal and distal convoluted tubular walls appeared unaffected compared to the control group (Figure 1).

The kidney histology of Group 3, administered dietary salt and subsequently treated with *T. tetraptera* (250 mg/kg), showed hypertrophied glomeruli, a reduced Bowman's capsular space, and less prominent parietal capsular cells. The proximal and distal convoluted tubular walls appeared unaffected compared to the control group. The kidney histology of Group 4, administered dietary salt and subsequently treated with *T. tetraptera* (500 mg/kg), showed a similar glomerular appearance, although with less prominent parietal capsular cells compared to the control. The kidney histology of Group 5, administered dietary salt and subsequently treated with Losartan, showed a similar glomerular appearance, although with less prominent parietal capsular cells compared to the control (Figure 1).

Collagen Distribution in the Kidney

Collagen is widely distributed in the kidney, within the interstitial matrix and glomerular basement membrane. The control group showed collagen distribution in the cortical interstitial matrix and glomeruli of the kidney. Group 2, administered dietary salt only, showed similar collagen distribution in the cortical interstitial matrix and glomeruli of the kidney compared to the control (Figure 2).

Group 3, administered dietary salt and subsequently treated with *T. tetraptera* (250 mg/kg), showed slightly less collagen distribution in the cortical interstitial matrix and glomeruli of the kidney compared to the control. Group 4, administered dietary salt and subsequently treated with *T. tetraptera* (500 mg/kg), showed slightly less collagen distribution in the cortical interstitial matrix and glomeruli of the kidney compared to the control. Group 5, administered dietary salt and subsequently treated with Losartan, showed slightly less collagen distribution in the cortical

interstitial matrix and glomeruli of the kidney compared to the control (Figure 2).

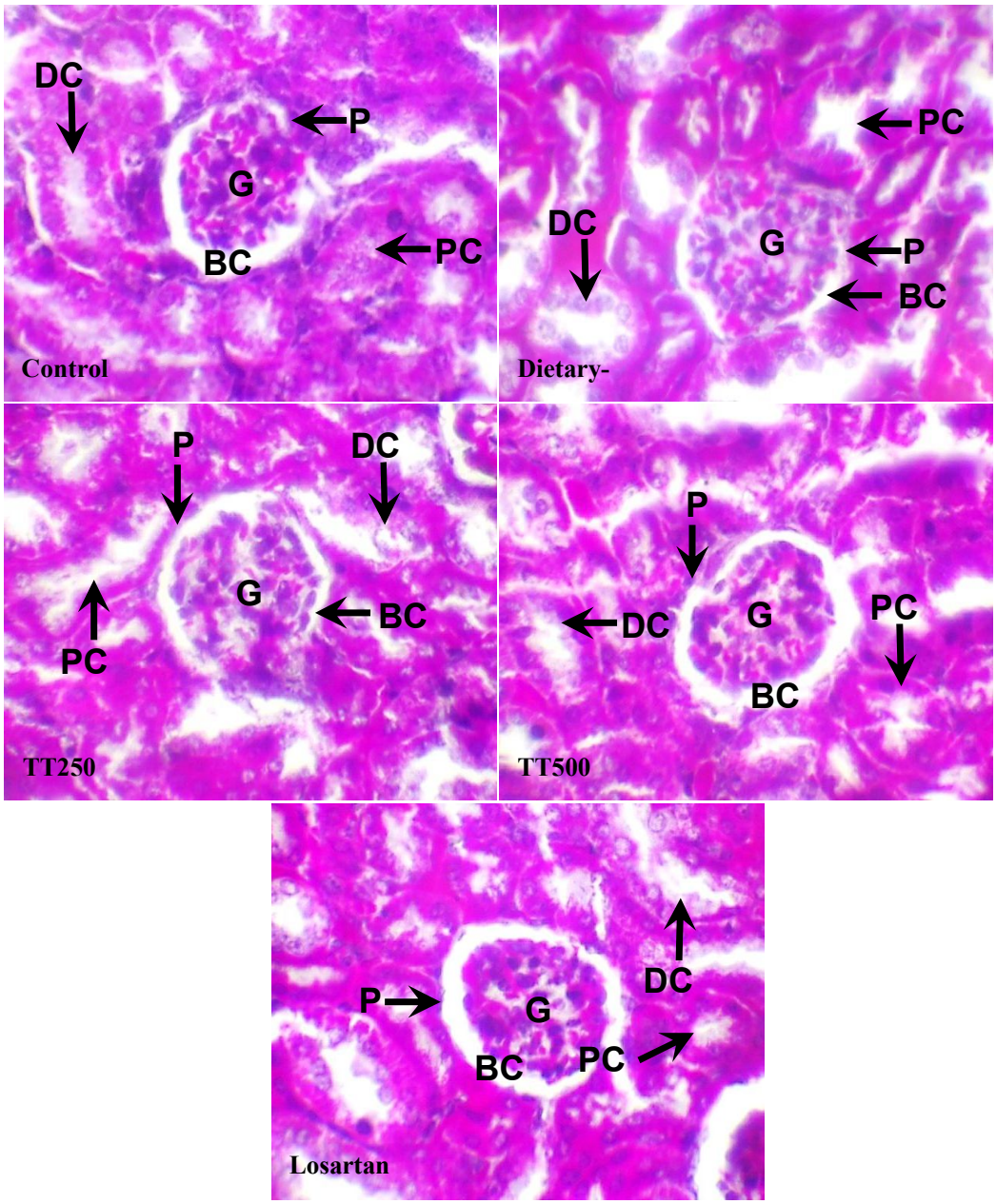

Fig. 1. Photomicrographs of the kidney cortical histology. CTR – control group shows a normal renal corpuscle. The dietary salt group shows a hypertrophied glomerulus (G) and reduced Bowman's capsular space (BC). TT250 – dietary salt and *T. tetraptera* (250 mg/kg) group shows a hypertrophied glomerulus (G) and reduced Bowman's capsular space. TT500 – dietary salt and *T. tetraptera* (500 mg/kg) group shows a normal renal corpuscle appearance. The Losartan group shows a normal renal corpuscle appearance. DCT – distal convoluted tubule; PCT - proximal convoluted tubule; P – parietal layer of Bowman's capsule; H. and E., ×400.

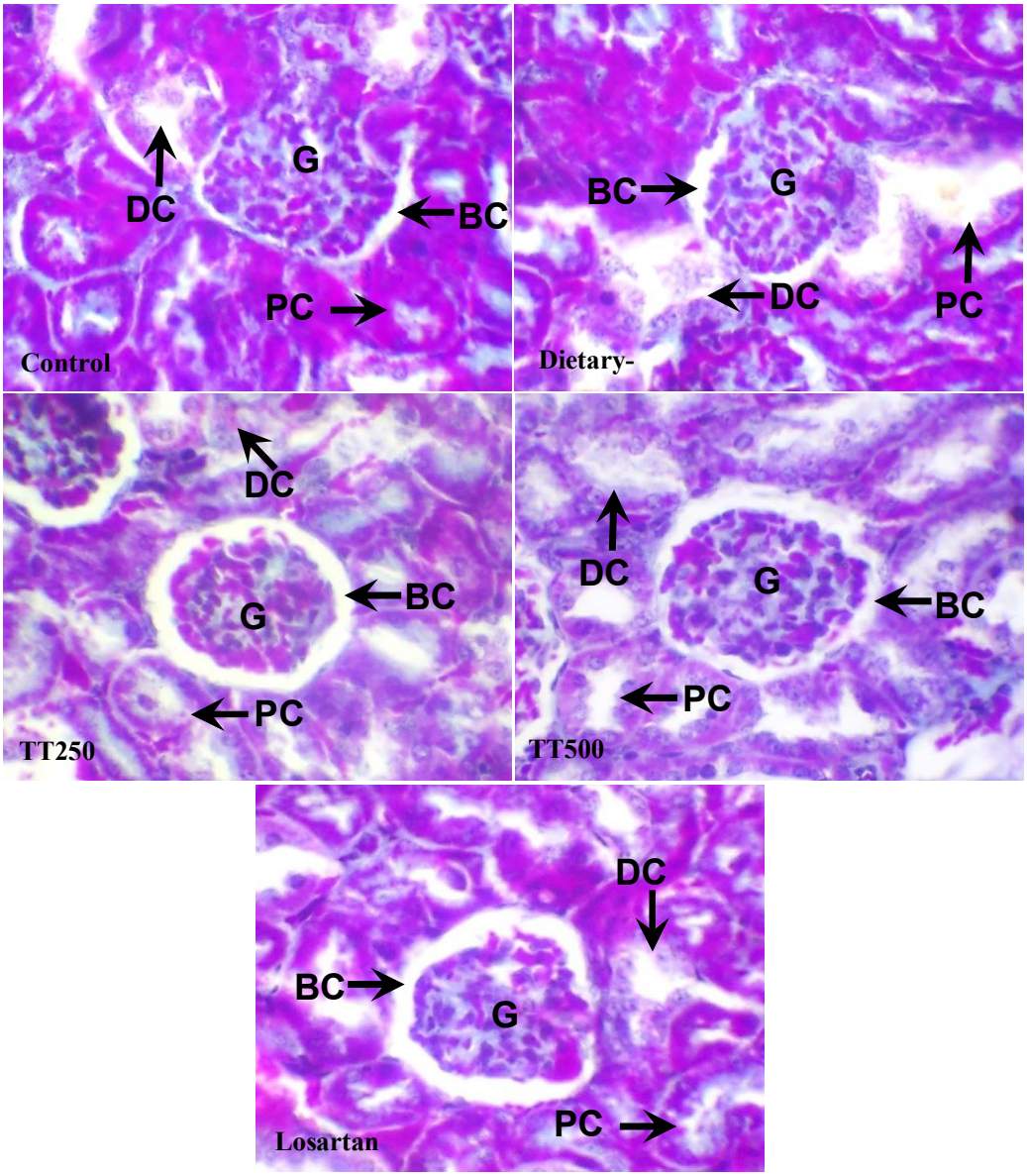

Fig. 2. Photomicrographs of the kidney cortical collagen distribution. CTR - control group shows collagen distribution in the cortical interstitial matrix and glomeruli of the kidney. Dietary-salt group shows slightly less collagen distribution. TT250 – dietary salt and *T. tetraptera* (250 mg/kg) group shows slightly less collagen distribution. TT500 – dietary salt and *T. tetraptera* (500 mg/kg) group shows slightly less collagen distribution. Losartan group shows slightly less collagen distribution. DCT – distal convoluted tubule; PCT - proximal convoluted tubule; P – parietal layer of Bowman's capsule; MT, ×400.

Discussion

The protective actions of *T. tetraptera* fruit ethanol extract were evaluated against the adverse effects of dietary salt on the kidneys of Swiss mice. *T. tetraptera* fruit showed protection of the kidney's structure and function.

Phytochemicals in plants benefit health by improving it and boosting immunity, and they are reported to have a wide range of pharmacological effects. Phytochemicals also show antioxidant activities (33). In the present study, phytochemicals in *T. tetraptera* ethanol fruit extract include flavonoids, phenols, and alkaloids, among others, which are similar to those reported in a previous study (34). These phytochemicals may be responsible for the plant's protective potency.

Changes in body weight are one means of assessing well-being (35). In the present study, there was body weight gain in the group administered dietary salt alone compared to the control. Body weight gain predisposes individuals to high blood pressure, increasing the risk of hypertension and exacerbating existing hypertension (36), and this may have been the case in the present study. The body weight result of the dietary salt-only group is at variance with that of Thierry et al. (24), who reported weight loss, possibly due to differences in animal species. There were also body weight gains in the groups administered dietary salt with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera,* and Losartan compared to the control, indicating that these interventions may not be antagonistic to metabolic processes. The 250 mg/kg and 500 mg/kg *T. tetraptera* groups showed body weight gains similar to those reported by Thierry et al. (24).

The kidney weights were not significantly different between the test groups (administered dietary salt only or with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera*, and Losartan) and the control. The organo-somatic indices were similar among the experimental groups. Kidney-somatic indices vary: Fregly (37) reported that the kidney-somatic index increases linearly with increasing blood pressure in rats. In the present study, this did not apply, which indicates differences in animal species or that hypertension may not have been fully attained.

The functionality of the kidney can be assessed by measuring its serum creatinine or electrolyte levels. Creatinine, a non-protein nitrogenous compound, is a by-product of muscle creatine conversion and is filtered mainly by the kidneys. This compound is excreted by glomerular filtration at a constant rate and is not influenced by diet or hydration. Changes in creatinine levels in the blood are associated with excretion and are therefore a reliable indicator of renal function (32). In the present study, the serum creatinine levels were not significantly different between the test groups (administered dietary salt only or with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera*, and Losartan) and the control, which may indicate no dysfunction of the kidneys. Serum creatinine increases with deficient glomerular filtration (38,39) but is reported unaffected until more than 50% of renal function is lost (40), which may have been the case in the present study. The creatinine results are similar to those of Thierry *et al*. (24), who also reported no difference between the test groups and the control.

There was, however, a significantly lower level of serum creatinine in the Losartan group compared to the dietary salt-only group, which supports its blood pressure-lowering action (41). It is reported that Losartan acts by inducing renal clearance of creatinine (42), resulting in decreased serum levels.

Electrolytes include ions of sodium, chloride, potassium, calcium, magnesium, and phosphorus, among others. In the present study, the serum sodium ion was significantly higher in the test groups administered dietary salt only, or with interventions of 250 mg/kg *T. tetraptera*, 500 mg/kg *T. tetraptera*, and Losartan compared to the control. A high-salt meal significantly increases serum sodium (9), which may have been applicable in the present study. High serum sodium leads to water retention, strain on the kidneys (9,10), and increases the risk of hypertension (43). Intervention with 250 mg/kg and 500 mg/kg *T. tetraptera* and Losartan did not reverse the high serum sodium level, indicating that blood pressure in these

groups was still high. These hypernatremia states may be due to the short duration of treatment, as most antihypertensive drugs are administered for prolonged periods.

There was no difference in serum chloride ion levels between the test groups administered dietary salt only or with interventions of 250 mg/kg and 500 mg/kg *T. tetraptera* compared to the control, indicating that chloride ion levels may not have been adversely affected. A decrease in serum chloride is associated with hypertension (44), which may not have been the case in the present study since no difference was observed. However, the serum chloride ion level was significantly lower in the test group administered dietary salt with the intervention of Losartan compared to the control.

There was no difference in serum potassium, calcium, phosphorus, and magnesium ions between the test groups compared to the control. However, the serum magnesium ion level was significantly lower in the group administered dietary salt only compared to the groups administered dietary salt with interventions of 250 mg/kg and 500 mg/kg *T. tetraptera*. The role of potassium, calcium, phosphorus, and magnesium ions in hypertension is not clear. In the body, magnesium may interact with other electrolytes, leading to the onset of high blood pressure. However, it is reported that hypertensive individuals show significantly decreased serum magnesium, calcium, and potassium (45), although this was not the case in the present study. Losartan acts by increasing urine flow and subsequent excretion of electrolytes (46), which explains its action on these electrolytes in the present study. Histologically, the kidneys of the dietary salt group showed hypertrophied glomeruli and reduced Bowman's capsules, indicating the hypertensive state of the animals. High salt intake is reported to induce glomerular damage (22,23), which may result from decreased effective renal blood flow, concomitant increased filtration fraction, and glomerular capillary pressure (16). The present result may also be due to this.

The dietary salt and *T. tetraptera* (250 mg/kg) group also showed hypertrophied glomeruli and reduced Bowman's capsules, indicating the state of the Swiss mice. The presence of these histopathological features may be due to the inability of *T. tetraptera* (250 mg/kg) to fully ameliorate the kidney effects of dietary salt. Although Thierry et al. (24) reported decreased blood pressure at a similar dose range, this may not automatically reverse the adverse kidney features observed in this study.

The dietary salt and *T. tetraptera* (500 mg/kg) group showed normal renal corpuscle appearance, indicating the ameliorating or protective effects of the plant extract. *T. tetraptera* ethanol extract protects against cyanide nephrotoxicity (46). This could be attributed to the plant's phytochemicals, which have organo-protective properties. The Losartan group showed normal renal corpuscle appearance, indicating its ameliorating or protective effects. Losartan protects against kidney cortico-medullary damage (44). This may have resulted in the normal renal corpuscle appearance in the present study.

The structure of tissues is maintained by their collagen distribution, which consists of bound amino acid complexes (47). These amino acid complexes are laid down by fibroblasts and myofibroblasts, initially known as procollagens (48). Masson's trichrome is a stain that highlights collagen fibers and is particularly used in the study of pathologies of the kidney and heart (9,30,49,50). In the dietary salt-only group, there was collagen distribution in the cortical interstitial matrix and glomeruli of the kidney, which indicates its hypertensive state. The kidney is reported to express mostly collagen types III and V in hypertension (51).

The 250 mg/kg and 500 mg/kg *T. tetraptera* groups showed slightly less collagen distribution, indicating their hypertensive states. *T. tetraptera* protects body tissues from injury and hypertension (24,25), and the kidney collagen deposition may have been reversed since the plant may have eliminated the trigger. The Losartan group also showed slightly less collagen distribution. Losartan inhibits angiotensin II-induced

hypertension (45), which may have contributed to this outcome.

T. tetraptera's protective actions are attributed to its phytochemicals, which have antioxidant and blood pressure-lowering properties, among others (24,25,46). The mechanism by which *T. tetraptera* effects its protection may not be unconnected with its antioxidants, although it is unclear how this works. The variations in the results of the present study could be due to two factors: alterations in blood pressure are individual-based, with some showing a higher sensitivity than others, hence the division of people into salt-sensitive and insensitive groups; and salt sensitivity increases with age, and since young adults were used, this may have played a role.

Conclusion

Dietary salt caused effects such as body weight gain, high serum sodium, adverse kidney histology, and collagen distribution, which suggest trauma. However, treatment with 250 mg/kg and 500 mg/kg of *T. tetraptera* showed potential for ameliorating or protecting against these adverse effects, with the *T. tetraptera* (500 mg/kg) treatment group showing better results.

Acknowledgments

We acknowledge Lydia Udoinyang for her assistance during the research and the staff of the animal facility for their support in animal handling.

Ethical statement

This study was conducted in accordance with the ethical standards outlined in the UU_FBMSREC_2022_002 code.

Data availability

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Funding/support

No funding was received for this research.

Author contributions

FME carried out the methods and collected the data; AUE supervised, critically analysed the data, and reviewed the manuscript; ANA supervised the research and reviewed the manuscript; MBE conceptualized the research and wrote the initial draft. All authors gave their consent for publication.

References

1. Breslin PA, Beauchamp GK. Salt enhances flavour by suppressing bitterness. Nature 1997;387(6633):563. http://dx.doi.org/10.1038/42388
2. Westphal G, Kristen G, Wegener W, Ambatiello P, Geyer H, Epron B, et al. Sodium chloride. In: Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH 2010. https://doi.org/10.1002/14356007.a24_317.pub4
3. Wu Q, Burley G, Li L, Lin S, Shi Y. The role of dietary salt in metabolism and energy balance: Insights beyond cardiovascular disease. Diabetes Obes Metab 2023; 25(5):1147–61.https://doi.org/10.1111/dom.14980
4. Chen SL, Dahl C, Meyer HE, Madar AA. Estimation of salt intake assessed by 24-hour urinary sodium excretion among Somali adults in Oslo, Norway. Nutrients 2018;10(7):900. http://dx.doi.org/10.3390/nu10070900
5. Htun NC, Suga H, Imai S, Shimizu W, Ishikawa-Takata K, Takimoto H. Dietary pattern and its association with blood pressure and blood lipid profiles among Japanese adults in the 2012 Japan National Health and Nutrition Survey. Asia Pac. J. Clin. Nutr 2018;27(5):1048–61. http://dx.doi.org/10.6133/apjcn.072018.04
6. World Health Organization. Massive efforts needed to reduce salt intake and protect lives. News release. World Health Organization 2023. https://www.who.int/news/item/09-03-2023-massive-efforts-needed-to-reduce-salt-intake-and-protect-lives
7. Turner MJ, Avolio AP. Does replacing sodium excreted in sweat attenuate the health benefits of physical activity? Int. J. Sport Nutr. Exerc. Metab 2016;26(4):377–89. http://dx.doi.org/10.1123/ijsnem.2015-0233.
8. Lucko AM, Doktorchik C, Woodward M, Cogswell M, Neal B, Rabi D, et al. Percentage of ingested sodium excreted in 24-hour urine collections: A systematic review and meta-analysis. J. Clin. Hypertens (Greenwich) 2018;20(9):1220–9. http://dx.doi.org/10.1111/jch.13353

9. Dickinson KM, Clifton PM, Burrell LM, Barrett PHR, Keogh JB. Postprandial effects of a high salt meal on serum sodium, arterial stiffness, markers of nitric oxide production and markers of endothelial function. Atherosclerosis 2014;232(1):211–6. http://dx.doi.org/10.1016/j.atherosclerosis.2013.10.032
10. Borrelli S, Provenzano M, Gagliardi I, Michael A, Liberti M, De Nicola L, et al. Sodium Intake and Chronic Kidney Disease. Int J Mol Sci 2020;21(13):4744. https://doi.org/10.3390/ijms21134744
11. Jung WJ, Park SM, Park JM, Rhee H, Kim IY, Lee DW, et al. Severe hypernatremia caused by acute exogenous salt intake combined with primary hypothyroidism. Electrolyte Blood Press 2016;14(2):27–30. http://dx.doi.org/10.5049/EBP.2016.14.2.27
12. Pfortmueller CA, Uehlinger D, von Haehling S, Schefold JC. Serum chloride levels in critical illness-the hidden story. Intensive Care Med. Exp 2018;6(1):10. http://dx.doi.org/10.1186/s40635-018-0174-5
13. Hancher J, Fisher J, Shenvi C. Severe hypernatremia and gastric dilation from chronic eating disorder and intentional salt ingestion. Am. J. Emerg. Med 2020;38(8):1700.e1-1700.e3. http://dx.doi.org/10.1016/j.ajem.2020.04.082
14. Shad ZS, Qureshi MSS, Qadeer A, Abdullah A, Munawar K, Khan MT, et al. Hyperchloremia in intensive care unit mortality: An underestimated fact. Cureus 2019;11(5):e4770. http://dx.doi.org/10.7759/cureus.4770
15. Sulyok E, Farkas B, Nagy B, Várnagy Á, Kovács K, Bódis J. Tissue sodium accumulation: Pathophysiology and clinical implications. Antioxidants (Basel) 2022;11(4):750. http://dx.doi.org/10.3390/antiox11040750
16. Campese VM. Salt sensitivity in hypertension. Renal and cardiovascular implications. Hypertension 1994;23(4):531–50. http://dx.doi.org/10.1161/01.hyp.23.4.531
17. Walkowska A, Kuczeriszka M, Sadowski J, Olszyñski KH, Dobrowolski L, Červenka L, et al. High salt intake increases blood pressure in normal rats: putative role of 20-HETE and no evidence on changes in renal vascular reactivity. Kidney Blood Press. Res 2015;40(3):323–34. http://dx.doi.org/10.1159/000368508
18. Rust P, Ekmekcioglu C. Impact of salt intake on the pathogenesis and treatment of hypertension. Adv. Exp. Med. Biol 2017;956:61–84. http://dx.doi.org/10.1007/5584_2016_147
19. Balafa O, Kalaitzidis RG. Salt sensitivity and hypertension. J. Hum. Hypertens 2021;35(3):184–92. http://dx.doi.org/10.1038/s41371-020-00407-1
20. Susin M, Mouradian J, Wilkes B. The kidney in hypertension: Pathology and pathogenesis. Hypertension in Kidney Disease Developments in Nephrology. Dordrecht: Springer; 1986. https://doi.org/10.1007/978-94-009-4271-4_2
21. Ferreira DN, Katayama IA, Oliveira IB, Rosa KT, Furukawa LNS, Coelho MS, et al. Salt-induced cardiac hypertrophy and interstitial fibrosis are due to a blood pressure-independent mechanism in Wistar rats. J. Nutr 2010;140(10):1742–51. http://dx.doi.org/10.3945/jn.109.117473.
22. Haruhara K, Tsuboi N, Koike K, Fukui A, Miyazaki Y, Kawamura T, et al. Renal histopathological findings in relation to ambulatory blood pressure in chronic kidney disease patients. Hypertens. Res 2015;38(2):116–22. http://dx.doi.org/10.1038/hr.2014.140
23. Gohar EY, Almutlaq RN, Daugherty EM, Butt MK, Jin C, Pollock JS, et al. Activation of G protein-coupled estrogen receptor 1 ameliorates proximal tubular injury and proteinuria in Dahl salt-sensitive female rats. Am. J. Physiol. Regul. Integr. Comp. Physiol 2021;320(3):R297–306. http://dx.doi.org/10.1152/ajpregu.00267.2020
24. Thierry BNM, Emery TD, Tom Esther NL, Bibi Farouck O, Claude BD, Theophile D. Protective effects of Tetrapleura tetraptera extract on high salt-induced hypertension in male rats. Int J Trop Med 8(2):54–61.
25. Kuate D, Kengne APN, Biapa CPN, Azantsa BGK, Abdul Manan Bin Wan Muda W. Tetrapleura tetraptera spice attenuates high-carbohydrate, high-fat diet-induced obese and type 2 diabetic rats with metabolic syndrome features. Lipids Health Dis 2015;14(1):50. http://dx.doi.org/10.1186/s12944-015-0051-0

26. Ekong MB, Bassey OO, Pessu NA, Kpobari GV, Okuku EI, Bassey RB, et al. Tetrapleura tetraptera Fruit Extracts Ameliorate Pentylenetetrazol-Induced Seizures as well as Ensuing Cognitive Deficit and Oxidative Stress. 2024 https://www.researchsquare.com/article/rs-3382014/v1
27. Akintola OO, Bodede AI, Ogunbanjo OR. Nutritional and medicinal importance of Tetrapleura tetraptera fruits (aridan). Afr J Sci Res 2015;4(6):36–41.
28. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011. https://www.ncbi.nlm.nih.gov/books/NBK54050/ doi: 10.17226/12910
29. Ekong M, Iniodu C, Essien IO, Edem S. Tetrapleura tetraptera (Schumach.) Taub. Fruit Extract Improves Cognitive Behaviour and Some Brain Areas of Pentylenetetrazol-Kindling Rats. Nig J Neurosci 2021 Dec 30;12(1):29–39.
30. Ekong FM, Ekanem AU, Aquaisua AN, Ekong MB. Tetrapleura Tetraptera Fruit Extract Protects Mice Heart Morphology from Chronic Dietary-Salt Intake. SciBase Hum Nutr Food Sci 1(1):1004.
31. IHC World. Novaultra Special Stain Kits. 2023. Masson's trichrome staining protocol for collagen fibers. https://www.ihcworld.com/_protocols/special_stains/masson_trichrome.htm
32. Molla MD, Degef M, Bekele A, Geto Z, Challa F, Lejisa T, et al. Assessment of serum electrolytes and kidney function test for screening of chronic kidney disease among Ethiopian Public Health Institute staff members, Addis Ababa, Ethiopia. BMC Nephrol 2020;21(1):494. http://dx.doi.org/10.1186/s12882-020-02166-0
33. Erukainure OL, Ebuehi OAT, Adeboyejo FO, Aliyu M, Elemo GN. Modulatory effect of fibre-enriched cake on alloxan-induced diabetic toxicity in rat brain tissues. Toxicol. Rep 2014;1:445–9. http://dx.doi.org/10.1016/j.toxrep.2014.07.010
34. Koma O, Olawumi O, Godwin E-U, Theophilus O. Phytochemical screening, in-vitro antimicrobial activity and antioxidant characteristics of tetrapleura tetraptera extracts. European J. Med. Plants 2016;17(2):1–10. http://dx.doi.org/10.9734/ejmp/2016/29585
35. Pan A, Kawachi I, Luo N, Manson JE, Willett WC, Hu FB, et al. Changes in body weight and health-related quality of life: 2 cohorts of US women. Am. J. Epidemiol 2014;180(3):254–62. http://dx.doi.org/10.1093/aje/kwu136
36. Huang Z, Willett WC, Manson JE, Rosner B, Stampfer MJ, Speizer FE, et al. Body weight, weight change, and risk for hypertension in women. Ann. Intern. Med 1998;128(2):81–8. http://dx.doi.org/10.7326/0003-4819-128-2-199801150-00001
37. Fregly MJ. Relationship between blood pressure and organ weight in the rat. Am. J. Physiol 1962;202:967–70. http://dx.doi.org/10.1152/ajplegacy.1962.202.5.967
38. Swedko PJ, Clark HD, Paramsothy K, Akbari A. Serum creatinine is an inadequate screening test for renal failure in elderly patients. Arch. Intern. Med 2003;163(3):356–60. http://dx.doi.org/10.1001/archinte.163.3.356
39. Ottka C, Vapalahti K, Määttä A-M, Huuskonen N, Sarpanen S, Jalkanen L, et al. High serum creatinine concentration is associated with metabolic perturbations in dogs. J. Vet. Intern. Med 2021;35(1):405–14. http://dx.doi.org/10.1111/jvim.16011
40. Hosten AO. BUN and creatinine. In: Clinical methods: The history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths; 1990. https://www.ncbi.nlm.nih.gov/books/NBK305/
41. L-Majed A, Assiri E, Khalil NY, Abdel-Aziz HA. Comprehensive Profile. Profiles of Drug Substances. Profiles of Drug Substances, Excipients and Related Methodology. 2015;40:159–94
42. Ivanov M, Mihailović-Stanojević N, Grujić Milanović J, Jovović Đ, Marković-Lipkovski J, Ćirović S, et al. Losartan improved antioxidant defense, renal function and structure of postischemic hypertensive kidney. PLoS One 2014;9(5):e96353. http://dx.doi.org/10.1371/journal.pone.0096353
43. Cole NI, Suckling RJ, Swift PA, He FJ, MacGregor GA, Hinton W, et al. The association between serum sodium concentration, hypertension and primary cardiovascular events: a retrospective cohort study. J. Hum. Hypertens

2019a;33(1):69–77. http://dx.doi.org/10.1038/s41371-018-0115-5

44. Nakajima K, Oda E, Kanda E. The association of serum sodium and chloride levels with blood pressure and estimated glomerular filtration rate. Blood Press 2016;25(1):51–7. http://dx.doi.org/10.3109/08037051.2015.1090711
45. Mulla S, Saddiqui WJ. Losartan. StatPearls [Internet] [Internet Treasure Island (FL): StatPearls Publishing.; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK526065/
46. Kadiri HE, Okoro IO, Ichipi-Ifukor PC. Tetrapleura tetraptera fruit protects against cyanide induced toxicity in rats. Iraqi J. Sci 2020;2504–14. http://dx.doi.org/10.24996/ijs.2020.61.10.7
47. Alexakis C, Maxwell P, Bou-Gharios G. Organ-specific collagen expression: implications for renal disease. Nephron Exp. Nephrol 2006;102(3–4):e71-5. http://dx.doi.org/10.1159/000089684
48. Mani K, Mani A. The significance of plasma collagen degradation products as biomarkers for advanced hypertensive heart disease. J. Clin. Hypertens. (Greenwich) 2021;23(5):1017–9. http://dx.doi.org/10.1111/jch.14205
49. Bargi R, Asgharzadeh F, Beheshti F, Hosseini M, Farzadnia M, Khazaei M. Thymoquinone protects the rat kidneys against renal fibrosis. Res Pharma Sci 2017;12(6):479.
50. Leonard AK, Loughran EA, Klymenko Y, Liu Y, Kim O, Asem M, et al. Methods for the visualization and analysis of extracellular matrix protein structure and degradation. Methods Cell Biol 2018;143:79–95. http://dx.doi.org/10.1016/bs.mcb.2017.08.005
51. Stanchev S, Stamenov N, Kirkov V, Dzhambazova E, Nikolov D, Paloff A. Differential collagen expression in kidney and heart during hypertension. Bratisl. Lek. Listy 2020;121(1):73–8. http://dx.doi.org/10.4149/BLL_2020_011

This is an open-access article distributed under the terms of the Creative Commons Attribution-noncommercial 4.0 International License which permits copy and redistribute the material just in noncommercial usages, as long as the original work is properly cited