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Tetrapleura Tetraptera Fruit Extract Protects Mice Heart Morphology from Chronic Dietary-Salt Intake

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Abstract

Dietary salt or sodium chloride is a common compound utilized for food and non-food functions. It is especially beneficial to the body because of its electrolyte composition which is utilized for several metabolic activities. But large stores of these electrolytes in the body are a prelude to cardiovascular problems. To manage such adverse effect on the heart, the present study investigated the role of *Tetrapleura tetraptera*, on the heart structure in mice. Twenty-five Swiss mice were grouped (n=5) as control, dietary-salt alone, or dietary-salt with *T. tetraptera* at 250 mg/kg and 500 mg/kg, and Losartan (50 mg/day). Administrations were by oral gavages, and lasted for eight weeks. Upon sacrifice, each of the hearts was weighed, and routinely processed for histology using haematoxylin and eosin, as well as collagen distribution using Mason's trichrome stains. The heart-body weight indices were not significantly different in the dietary salt group, while the histology showed increased myocardial fibres and nuclei hypertrophy as well as deepened collagen deposits compared with the control. The groups with interventions of *T. tetraptera* (250 mg/kg and (500 mg/kg) and Losartan showed normal myocardial fibres and collagen deposits compared with the control. These results indicate that 250 mg/kg and 500 mg/kg *T. tetraptera* fruit extract treatment have some protection on the heart.

Keywords: Tetrapleura Tetraptera; Myocardium; Sodium chloride; Histology; Collagen.

Introduction

Dietary-salt or sodium chloride is utilized in almost every food items, either as an added item, or naturally inherent in them, to bring out the taste and flavour [1,2]. Dietary salt is mostly sourced from the sea water, with other natural sources adding a very small amount [2].

Sodium and chloride ions are two important body electrolytes derived from dietary-salt, which are essential for the numerous metabolic activities of the body [3]. Their accumulation in high amount in the body is associated with increased intake or a diseased state that enables their retention [4,5]. A build-up of these electrolytes is implicated in high blood pressure which affects the heart, with ventricular atrophy and blood vessels damage reported [6-8].

The heart is an important organ that distributes blood throughout the body, and as such impairment of its function can lead to death [9]. To prevent such, several drugs are in the market to lower blood pressure [10]. However, their use may not help if the wrong drug family with a different mechanism of action is administered.

Studying hypertension and the screening of potential antihypertensive agents cannot be achieved if the heart reaction to them is not put in perspective. Some plant materials have been reported to modulate blood pressure among other actions. *Tetrapleura tetraptera* (*T. tetraptera*), a member of the Fabaceae family is one of such plants reported in blood pressure regulation and provide other health benefits in experimental animals [11-13]. This plant is common in Nigeria as aidan in Yoruba and uyayak in Ibibio, where they are utilized spice and dietary supplements [14]. It is possible that *T. tetraptera* acts

through the renin-angiotensin pathway, thereby restoring the kidney function, and eventually blood pressure, since this pathway is elicited by dietary salt [8]. This study evaluated the action of *T. tetraptera* fruit extract on the heart of mice following dietary salt intake.

Materials and methods

Plant and drug sourcing and preparations

Mature fruits of *T. 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 of the University of Uyo, with the voucher number UUPH/A32 (f) assigned. The fruits were air-dried, pulverised, and extracted in 80% ethanol for 72 h. The extract was filtered, evaporated to dryness, and preserved at 4℃. The *T. tetraptera* extract was reconstituted daily in distilled water, and orally administered to the animals at 250 mg/kg and 500 mg/kg body weight.

Sodium chloride were obtained as dietary-salt from a local market in Uyo Metropolis of Nigeria, stored and protected from moisture in an airtight container at room temperature. The salt was dissolved distilled water, with a fresh solution of 9% volume/volume of dietary-salt prepared daily. Losartan obtained from a reputable Pharmacy in Uyo, Nigeria was also dissolved in distilled, and the equivalent body weight dose administered.

Animal handling

Twenty-five adult male mice (25-30 g) were obtained from the animal house of the Faculty of the Basic Medical Sciences, University of Uyo, Nigeria. The mice were kept in clean cages and acclimatized for 14 days under natural 12 h light/dark cycle and temperature of 26-29℃. The mice were allowed standard mice 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 mice were handled following the guidelines for use of animal for laboratory research [15].

Experimental design

The mice were grouped as: Control (10 mL/kg of distilled water), dietary-salt in distilled water (9% v/v or 90 mg/mL), and dietary-salt (90 mg/mL) with interventions of *T. tetraptera* (250 mg/kg), *T. tetraptera* (500 mg/kg) and Losartan (50 mg/day). All the administrations were by oral gavages, and lasted for eight weeks (four weeks of dietary-salt-only, and four weeks of intervention concomitantly with the dietary-salt). The body weights were measured weekly.

Termination of the experiment

The mice were anaesthetized with 50 mg/kg body weight ketamine hydrochloride (i.p.) after overnight fasting, and sacrificed. The hearts were collected through an incision in the thoraco-abdominal wall, weighed and fixed in 10% buffered formalin. The fixed hearts were routinely processed for paraffin wax embedding, sectioned at 8 um thickness and further processed for histology using haematoxylin and eosin technique, and histochemistry using Masson's trichrome technique [16].

Statistical analysis

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

Results

Body weight effect of T. *Tetraptera*

The body weight of mice in the Losartan group was significantly higher compared with the control. The body weight of mice in the groups administered dietary-salt alone or with interventions of 250 mg/kg and 500 mg/kg of *T. tetraptera* were significantly less compared to the Losartan group (Table 1).

Organ weight and organo-somatic indices effect of *T. tetraptera*

The heart mean 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 (Table 1).

Effect of *T. tetraptera* on the histology of the heart

The heart wall consists of three layers: an inner endocardium, a middle myocardium, and an outer epicardium. In the present study focus was on the myocardium, which consists of myocardial fibres. In the control section, the myocardium showed normal longitudinal myocardial fibres and their connections through intercalated disks (Figure 1).

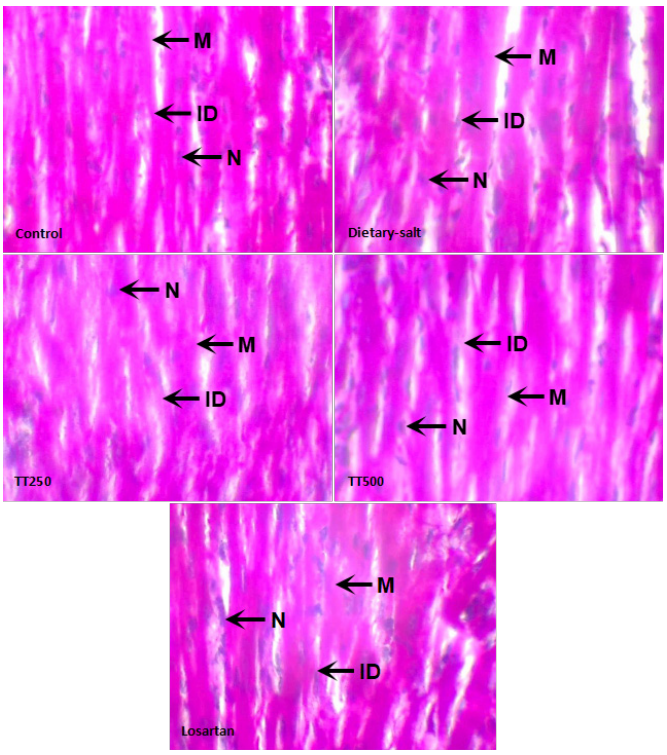

Figure 1: Photomicrographs of the heart histology showing the myocardium. CTR - control shows normal myocardial fibres (M). Dietary-salt group shows increased myocardial fibres (M) size and hypertrophied nuclei (N). TT250 - dietary-salt and *T. tetraptera* (250 mg/kg) group shows normal myocardial fibres size as the control. TT500 - dietary-salt and *T. tetraptera* (500 mg/kg) group shows normal myocardial fibres size as the control. Losartan group shows normal myocardial fibres size as the control. ID - intercalated disks; H. and E., ×400.

Table 1: Body and organ weights and organo-somatic indices.

Group (n = 5)	Body Weight (g)	Heart Mean Weight (g)	Heart-Somatic Index
Control	28.00±0.55	0.13 ± 0.01	0.46
Dietary-salt	28.20 ±0.66[a]	0.12 ± 0.00	0.43
Dietary-salt and *T. tetraptera* (250 mg/kg)	28.40±0.87[a]	0.12 ± 0.01	0.42
Dietary-salt and *T. tetraptera* (500 mg/kg)	30.20±0.97[a]	0.13 ± 0.01	0.43
Dietary-salt and Losartan	32.80±0.49**	0.13 ± 0.01	0.40

ANOVA and Tukey post hoc test.
Data are presented as Mean ± Standard error of mean.
** - Significantly different from the control at $p < 0.05$.
a - Significantly different from the Losartan group at $p < 0.05$.

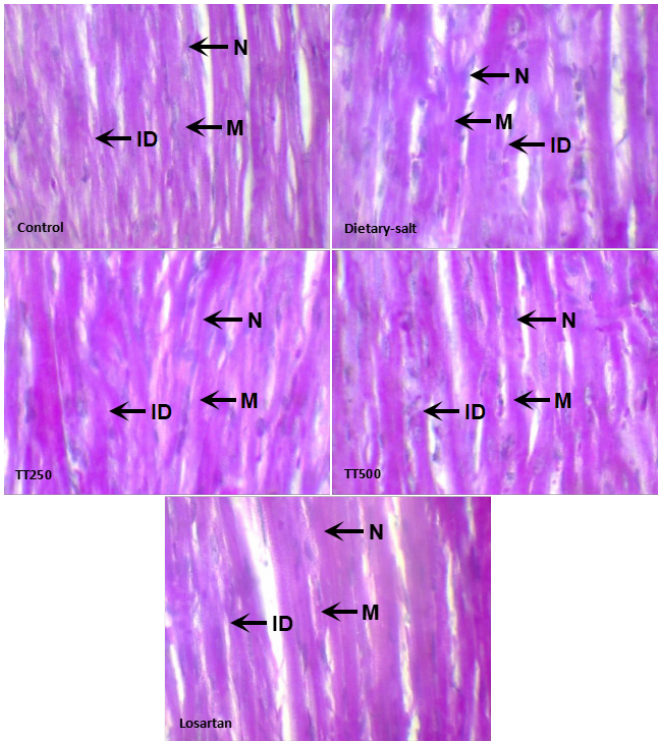

Figure 2: Photomicrographs of the heart histology showing the myocardium. CTR - control shows normal myocardial fibres (M). Dietary-salt group shows increased myocardial fibres (M) size and hypertrophied nuclei (N). TT250 - dietary-salt and *T. tetraptera* (250 mg/kg) group shows normal myocardial fibres size as the control. TT500 - dietary-salt and *T. tetraptera* (500 mg/kg) group shows normal myocardial fibres size as the control. Losartan group shows normal myocardial fibres size as the control. ID - intercalated disks; H. and E., ×400.

In the group administered dietary salt alone, the heart myocardium showed increased muscle fibres and hypertrophied nuclei compared with the control. In the groups administered dietary-salt and subsequently treated with *T. tetraptera* (250 mg/kg and (500 mg/kg), showed normal myocardial fibres was observed. The group administered dietary-salt and subsequently treated with Losartan, showed similar normal myocardial fibres, all compared with the control (Figure 1).

Effect of *T. tetraptera* on collagen distribution in the heart

Collagen is distributed in the heart myocardium: In the control group, collagen surrounds myofibrillar bundles. The group administered dietary-salt-only, showed more deposits of collagen as expressed in increased staining compared with the control. The group administered dietary-salt and subsequently treated with *T. tetraptera* (250 mg/kg), showed more deposits of collagen compared with the control. The group administered dietary-salt and subsequently treated with *T. tetraptera* (500 mg/kg), showed more deposits of collagen compared with the control. The group administered dietary-salt and subsequently treated with Losartan, showed more deposits of collagen compared with the control (Figure 2).

Discussion

This study aimed to investigate the ameliorative effect of *T. tetraptera* fruit extract against dietary salt adverse actions on the heart of Swiss mice. The results indicated that *T. tetraptera* fruit extract showed some protection on the heart against dietary salt.

The results showed that the heart-somatic indices were similar among the experimental groups. Fregly [17] reported that the heart-somatic index is sigmoid in rats. In the present study, this did not apply, which indicates animal species difference, or possible only after a protracted administration. Thus, the overall size of the heart may not be affected in chronic dietary salt intake.

Histologically, the mice hearts in the dietary-salt group showed increased myocardial fibres and nuclei hypertrophy. Cardiac muscle hypertrophy is one of the hallmarks of heart damaged in hypertension [6,18], which may have been the case in the present study. Increase salt intake induces cardiac muscle hypertrophy probably through increased angiotensin II myocardial concentrations [14].

In the 250 mg/kg and 500 mg/kg *T. tetraptera* groups, the myocardial fibres appeared normal, indicating the ameliorative or protective action of *T. tetraptera* on the heart. The heart of the Losartan group also showed normal myocardial fibres, indicating its ameliorative and protective activities. Losartan relieves hypertension, which is soothing to the heart [9,19].

The structure of tissues is maintained by its collagen distribution [20], which is very important to the heart, as it provides a structural framework to cardiac myocytes, impart stiffness to the myocardial wall and aid force transmission [21]. Histochemically, Masson's trichrome highlights collagen fibres, an important trio of stains for studying pathologies of the heart [22-24]. In the dietary salt group, there was an increased collagen deposit around the myofibrillar bundles. Hypertrophy of the heart myocardium is often accompanied by stiffness, which may arise from excessive collagen deposition [25]. Collagen types I and V are expressed in the heart myocardium during pathological conditions such as hypertension [26]. Excess cardiac collagen type I synthesis and deposition may be involved in the enhancement of myocardial fibrosis that accompanies the development of heart failure in hypertensive heart disease [27].

In the mice treated with 250 mg/kg and 500 mg/kg *T. tetraptera* extract, there were also collagen deposits in the myofibrillar bundles. *T. tetraptera* protects body tissues from injury, and the collagen deposition may not be adverse in this case [10,11]. The Losartan group also showed collagen deposits. Angiotensin II stimulates collagen production [28], and Losartan reversibly and competitively prevents angiotensin II binding to the angiotensin I receptor in tissues. Thus, Losartan inhibits the angiotensin II-induced cardiac remodelling [9,19], which may have played out in the present study.

The protective action of *T. tetraptera* is often attributed to its phytochemicals, as they show blood pressure lowering properties, among other protective actions [10,11,29]. The mechanism of *T. tetraptera* protective action may not be unconnected with its antioxidant properties, which most disease condition compromise. And since dietary salt elicits increased blood pressure through the renin-angiotensin pathway [8], it is possible that the reported *T. tetraptera* action may follow similar mechanism.

Conclusion

Dietary-salt did not affect heart-body weight index, but showed adverse histology and collagen deposits. However, 250 mg/kg and 500 mg/kg *T. tetraptera* fruit extract treatment ameliorated the adverse myocardial fibres histology, which appeared better in the *T. tetraptera* (500 mg/kg) treatment group.

Declarations

Financial Support: No funding was received for this research.

Conflict of Interest: Authors do not declare any conflict of interest.

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