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Original article

Tender coconut water maintains the level of electrolytes and renin in fructose-fed hypertensive rats

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ABSTRACT

Studies on fructose induced hypertension have suggested that imbalance in electrolytes and renin angiotensin-aldosterone system play an important role in the etiology. The present study evaluated the effect of Tender coconut water infusion on serum electrolytes, plasma L-Arginine, plasma renin activity and aldosterone levels on fructose-fed hypertensive rats. Male albino Sprague Dawley rats were fed with high fructose diet (71%) and TCW was administered by gastric intubation (4ml/100g of body weight). The results showed that serum concentration of sodium was significantly ($p<0.05$) higher and serum potassium, calcium and magnesium were significantly lower in fructose-fed hypertensive rats compared to control which was mitigated on treatment with Tender coconut water. Concentration of plasma L-Arginine lowered, serum nitrite in fructose fed rats were increased by tender coconut water supplementation. Plasma renin activity and aldosterone also increased in fructose fed rats, was also lowered by the administration of Tender coconut water. Histopathological analysis of the kidney showed that Tender coconut water supplementation reverted the congestion and hypercellularity of glomerular tuft induced by high fructose diet. The study revealed that Tender coconut water was found to exert beneficial effects on electrolyte imbalance and elevated plasma renin activity and aldosterone level in fructose fed hypertensive rats.

1. Introduction

Hypertension is a major health problem worldwide. Lifestyle factors such as diet play an important role in the etiology of essential hypertension. Increased dietary carbohydrate intake can raise blood pressure in experimental animals and provide an acquired form of systolic blood pressure [1]. Several mechanisms have been proposed for fructose-induced hypertension namely baroreceptor reflex, renin-angiotensin-aldosterone system, alteration of extra cellular fluid volume, activation of sympathetic nervous system and abnormal function of vascular endothelium [2]. Insulin resistance and glucose intolerance are common features of hypertension in humans and in animal models [3]. Hyperinsulinemia was thought to cause hypertension because it led to sodium retention, sympathetic nerve activation, and vascular smooth muscle cell proliferation [4]. Sympathetic activation stimulates renin secretion and the resulting Angiotensin II, which further activates the sympathetic nervous system [5]. Angiotensin II increases the aldosterone secretion; it causes vasoconstriction through an increase in calcium influx [6]. Electrolytes play a central role in blood pressure regulation and sodium retention has been reported to be one of the causes for hypertension [7]. The Dietary approaches to stop hypertension (DASH) studies demonstrated that a well balanced diet, rich in antioxidants including Vitamin C and E was effective in lowering blood pressure in both hypertensive and normotensive people [8] by reducing aldehyde conjugate formation and oxidative stress, by improving insulin resistance and endothelial function, by normalizing calcium channels and peripheral vascular resistance [3].

Recently there has been focus on blood pressure lowering effects of dietary plants. Epidemiological studies suggest that higher intake of potassium, calcium, magnesium [9], peptides from fish or milk proteins [10], antioxidants [11], polyphenols[12], polyunsaturated fatty acids[13], and food components[14] are beneficial for preventing hypertension and cardiovascular diseases.

Tender coconut water (TCW) is the natural nutritious wholesome beverage and possesses a series of nutritional and

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therapeutic properties. The richness of macro and micro nutrients in TCW are reported to have hypolipidemic[15], cardioprotective, antihypertensive and antioxidant effects[16, 1, 17].

Dietary supplementation with antioxidants may be beneficial, inexpensive, first-line alternate treatment modality for hypertension without any side effects. Thus the present study was undertaken to find out the effect of TCW on electrolyte imbalance, plasma renin activity and aldosterone levels in fructose fed hypertensive rats.

2. Materials and Methods

2.1. Tender coconut water (TCW)

Young coconuts (Cocos nucifera) (West Coast Tall Variety), 5-6 months of age harvested from the coconut trees grown on the University campus were used for the study. The coconuts were dehusked, broken carefully and liquid endosperm was collected and used for the experiment.

2.2. Animals

Male Sprague- Dawley rats weighing between 170 -190 g, were used for the study. The rats were kept in a laboratory animal unit with a 12 hr light / dark cycle. Throughout the experiment, room temperature was maintained at 25 ± 1˚C. The rats were maintained on a standard chow diet (Sai Feeds, Bangalore, India) and water ad libitum prior to dietary manipulation. Institutional ethical committee approved the study and the guidelines for care and use of laboratory animals published by the US National Institute of Health (NIH Publication no. 85-23, revised1996) [18] were followed throughout the experimental period.

After acclimatization, the experimental rats were randomly divided into 4 groups of 6 rats each. The first and second group of rats received the control diet containing 71% cornstarch, 8% fat and 16% protein. The third and fourth group received a diet containing 71% fructose, 8% fat and 16% protein. The duration of experimental period was five weeks.

Group I-control
Group II-control + TCW (4 ml/ 100 g body weight).
Group III- Fructose-fed
Group IV- Fructose-fed + TCW (4 ml/ 100 g body weight).
From the thrid week onwards rats of group II and IV recieved TCW by gastric intubation. All other rats recieved same volume of distilled water.

At the end of the experiment, the animals were sacrificed by decapitation and blood samples were collected for biochemical determinations. Serum sodium and potassium was assayed [19] by a kit purchased from M/S Excel diagnostics Pvt. Ltd. Hyderabad, India. Serum calcium was assayed [20] using a kit from ERBA diagnostics, Mannheim, Germany and Magnesium [21] by kit purchased from Wako Diagnostics, Japan. Plasma L-Arginine [22], serum nitrite [23], Plasma renin[24] and plasma aldosterone[25] were measured in all the groups. Histological studies of kidney were also done in control and experimental groups [26].

2.3. Statistical analysis

The SPSS statistical program was employed for statistical analysis. The results were evaluated using analysis of variance (ANOVA) utilizing the F test. The results are presented as the mean value ± SD for the control and experimental rats. Differences among the means for the groups were assessed using the Duncans Multiple Range Test to determine which mean values were significantly different at $p<0.05$.

3. Results and Discussion

Several studies have found that high fructose diet produce an animal model of hypertension reported earlier [1, 27]. Electrolyte imbalance and alteration in renin-angiotensin system plays a key role in the pathogenesis of hypertension. Numerous epidemiological and intervention studies have demonstrated a positive correlation between sodium intake and elevated blood pressure [28]. Mineral micronutrients viz., Sodium (Na), Potassium (K), Calcium (Ca) and Magnesium (Mg) have been shown to have a direct effect on blood pressure based on epidemiological, laboratory and clinical investigations [2]. Dietary intake of K, Ca and Mg has been reported to lower blood pressure. Combinations of these mineral supplements would lower blood pressure, which would be greater than the reductions reported in studies of cation alone [29]. In high fructose-fed group the serum concentrations of K, Ca and Mg were found to be significantly ($p<0.05$) lower and serum Na was found to be higher which was reversed on treatment with TCW (Table 1).

Table 1. Demographic characteristics of different study group.

Sodium (mMol/l)	Potassium (mMol/l)	Calcium (mg/dl)	Magnesium (mg/dl)
123.21±4.35^{b}	5.3±0.3^{b}	12.48±0.4^{b}	3.46±0.3^{b}
122.21±4.6^{b}	5.5±0.17^{b}	12.9±0.3^{b}	3.78±0.27^{b}
135.71±3.1^{a}	3.05±0.4^{a}	9.5±0.5^{a}	1.7±0.2^{a}
129.4±1.4^{b}	4.96±0.3^{b}	11.8±0.4^{b}	3.3±0.2^{b}

Values are mean ±SD for six rats.
P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 3.

In the study, increased concentration of sodium in fructose fed rats, which was lowered by tender coconut water treatment. Variation in Na intake will lead to changes in insulin sensitivity and plasma insulin concentration [30]. Hyperinsulinemia in high fructose fed rats is shown to be associated with renal sodium retention [31], which could contribute to the development of hypertension. The renin angiotensin system (RAS) plays a central role in the regulation of blood pressure, electrolyte and volume homeostasis. It acts primarily by controlling renal reabsorption of sodium. Renin is a proteolytic enzyme which when released initiates a cascade of enzyme reactions leading to the production of Angiotensin II having many actions viz., systemic vasoconstriction, vasopressin release, increased sodium reabsorption and aldosterone release. RAS determines the homeostasis of Na and K [32]. Plasma aldosterone levels and renin activity were elevated in fructose-fed rats, which were restored, to normal by TCW treatment (Table 2).

Table 2. Activity of plasma renin and aldosterone

Renin (ng/ml/hr)	Aldosterone (ng/dl)
3.34±0.007^{b}	189.0±1.4^{b}
3.21±0.12^{b}	188.7±1.0^{b}
6.1±0.14^{a}	222.5±3.5^{a}
3.4± 0.007^{b}	197±1.4^{b}

Values are mean ±SD for six rats.
P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 3.

Vascular RAS upregulated in fructose fed a rat, which indicates that vascular RAS mediates vascular dysfunction and vascular oxidative stress in fructose fed hypertensives [33]. Aldosterone is reported to increase the blood pressure [34] and plays an important role in the control of blood pressure and water and electrolyte homeostasis. Excess mineralocorticoids cause sodium and water retention and potassium excretion by the kidney followed by "sodium escape" with the maintenance of the new volume associated with hypertension and hypokalemia[35].

Chemical characterization of TCW (Table 3) shows that it is a rich source of several biologically active components namely potassium (300 mg/dl), calcium (40 mg/dl), magnesium (16 mg/dl) Vitamin C (30mg/dl) and free aminoacid L-Arginine (30 mg/dl). L-Arginine is reported to have significant hypolipidemic, antihypertensive and antiatherogenic effect [36, 37]. Potassium decreases urinary calcium excretion and increases body calcium balance, probably by increasing renal calcium resorption[38]. Potassium supplementation has also been reported to lower systolic and diastolic blood pressure [39]. High potassium diets have been reported to prevent hypertensive endothelial injury and intimal thickening [40]. The direct effect of K on blood pressure regulation would be through its effect on natriuresis, baroreceptor sensitivity, the RAS system, vasodilation, and sympathetic nervous system activation. In addition to this, K is also involved in the inhibition of free radical formation, vascular smooth muscle proliferation and arterial thrombosis [41,42].

Table 3. Composition of tender coconut water (TCW) used for the study

Constituents	TCW
Total sugar (%)	4.6
Total protein (mg/dl)	146
L-Arginine (mg/dl)	32
Vitamin C (mg/dl)	30
Polyphenols (mg/dl)	3.75
Selenium (mg/dl)	0.001
Sodium (mg/dl)	40
Potassium (mg/dl)	300
Magnesium (mg/dl)	16
Calcium (mg/dl)	40

Ca supplementation has also been shown to decrease blood pressure in hypertension [43]. NO synthase produces NO from L-arginine through Ca dependent process. NO is responsible for the acetylcholine mediated vascular relaxation. Accelerated degradation of NO may lead to impaired vasodilation and hence increased blood pressure [44]. Several studies have indicated that magnesium plays a role in the etiology of hypertension and in glucose homeostasis. Magnesium administration, concomitant with potassium, assists tissue replenishment of potassium [45]. The role of Ca in regulating vascular tone is dependent on magnesium. Mg deficiency have been reported to increase the susceptibility of lipoproteins to lipid peroxidation [46] which results in a high rate of free-radical formation [47] which inactivate the endothelium-derived relaxation factor, NO, which when undergo degradation by superoxide anions could contribute to the enhancement of the arterial contractile response, resulting in the development and maintenance of hypertension [48]. From the results it is clear that, sodium retention occurs in fructose fed hypertensive rats, which alters the electrolyte balance and renin angiotensin homeostasis. Tender coconutwater possess significant antihypertensive property that maintains the electrolyte balance and renin aldosterone homeostasis.

Tender coconut water supplementation increased the plasma L-Arginine and serum nitrite levels compared to fructose fed control rats.

(Table 4). L-Arginine infusion has shown to produce peripheral vasodilation and have antihypertensive effects [49]. Dietary supplementation with L-Arginine has been reported to reverse dysfunctional arginine/nitric oxide pathway in endothelium [50]. Increased plasma L-Arginine and serum nitrite indicate increased production of nitric oxide in tender coconut water supplemented rats. NO synthase produces NO from L-Arginine through calcium dependent process. NO is responsible for the acetylcholine mediated vascular relaxation. Accelerated degradation of NO may lead to impaired vasodilation and hence increased blood pressure [44].

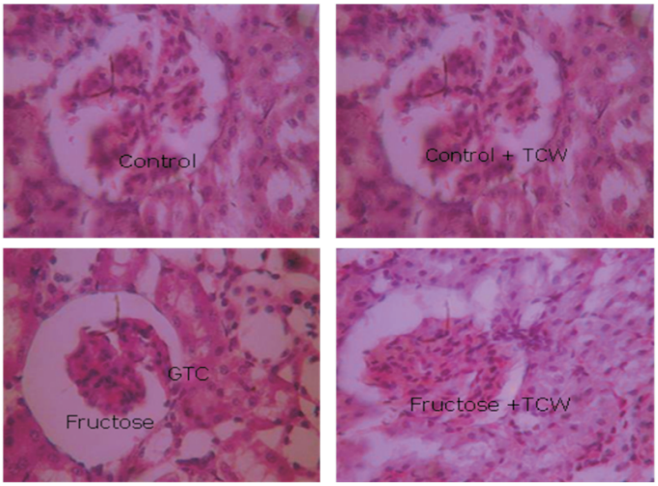
Table 4. Concentration of plasma L-arginine (μ mol/ml) and serum nitrite (μ mol/l)

Plasma L-Arginine	Serum nitrite
0.132 ± 0.002^{b}	11.65 ± 0.88^{b}
0.135 ± 0.002^{b}	11.91 ± 0.76^{b}
0.125 ± 0.004^{a}	10.24 ± 0.27^{a}
0.183 ± 0.013^{b}	11.97 ± 0.72^{b}

Values are mean ± SD for six rats .
P< 0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 3.

Kidney plays a central role in the regulation of the balance of body salt and water, and disordered regulation of renal functions is responsible for the altered balance of salt and water in pathophysiological states including some experimental models of hypertension. Histopathological studies of kidney revealed that high fructose diet produced glomerular tuft congestion (Fig. 1), which was reverted by TCW treatment. This suggests that renal dysfunction could be ameliorated by treatment with TCW, which may be beneficial in the control of blood pressure.

Figure 1. Light microscopic appearance of the kidney sections stained with Hematoxylin-Eosin (40X)



1.Control Renal glomeruli shows normal structure. The renal tubules lined with low simple cubic epithelium. The tubules have a relatively regular distinct lumen.

2. Control + TCW- The kidney architecture same as that of control. No abnormal features.

3. Fructose fed rats High Fructose diet produced glomerular tuft congestion (GTC), thickening and hypercellularity of glomerular tuft. Degeneration of tubular epithelia.

4. Fructose fed rats + TCW- Congestion and thickening of glomerular tuft is mildly reverted.

4. Conclusion

In conlcusion, the results of the present study revealed that altered activity of renin angiotensin in fructose fed rats is reversed by tender coconut water which restored the electrolyte imbalance almost normal. Tender coconut water also improved renal dysfunction, which was evident from the histopathological analysis that tender coconut water could enhance the electrolyte imbalance and renin angiotensin system, which plays an important role in the pathophysiology of hypertension.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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