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Aqueous Root Extract of *Citropsis articulata* Reduces Mean Arterial Blood Pressure and Serum Sodium Levels but has No Effect on Serum Potassium and Calcium Levels in Deoxycorticosterone Acetate Salt-Induced Hypertensive Male Wistar Rats

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Abstract

Background: Across the globe, about 26% of the world's populace suffers from hypertension, and it is projected that this figure will rise to 29% by 2025. This increase is primarily attributed to the growing incidence in economically developing countries. The elevated prevalence of hypertension imposes a significant public health burden on a global scale, especially in developing nations such as Uganda. The current study assessed the effects of aqueous root extract of *Citropsis articulata* on mean arterial blood pressure and serum levels of electrolytes (sodium, potassium & calcium) in Deoxycorticosterone acetate (DOCA) salt-induced hypertensive male Wistar rats.

Methods: Twenty-four male Wistar rats were employed and put into six groups (n = 4). Group I had no hypertension induced and these were given standard feed only. Hypertension was induced using Deoxycorticosterone acetate (DOCA) salt and 1ml of sodium chloride (NaCl) in drinking water for groups II, III, IV, V & VI. Groups III, IV & V were orally administered with aqueous root extract of *Citropsis articulata* at doses of 200, 400, 600 mg/kg respectively while group VI was treated with Propranolol at 1mg/kg. Blood pressure was measured using a power lab non-invasive blood pressure machine while serum electrolytes (sodium, potassium, calcium) were measured using the Ion selective electrode method.

Results: The aqueous root extract of *Citropsis articulata* significantly reduced the mean arterial blood pressure and serum sodium levels but did not have a significant effect on serum potassium and calcium levels.

Conclusion: The study showed that the aqueous root extract of *Citropsis articulate* reduced the mean arterial pressure and serum sodium levels in DOCA-induced hypertensive male Wistar rats.

Introduction

Hypertension is a chronic medical ailment with marked increase in blood pressure that exceeds 140/90 mm of Hg in three consecutive measures [1]. Almost 1.13 billion people worldwide were suffering from hypertension by 2014 [1]. The general occurrence of elevated blood pressure in Africa was 19.7% in 1990 and 27.4% in 2000, and 30.8 percent in 2010, with a combined rate of realization of 16.9%, 29.2%, and 33.7 percent, respectively, in 1990, 2000, and 2010. Based on a 1990 study, it was projected that the prevalence of hypertension would affect 54.6 million individuals, with subsequent estimates of 92.3 million in 2000, 130.2 million in 2010, and a forecasted elevation to 216.8 million cases by 2030 [2]. In a study conducted in Uganda, it was found out that hypertension was quite common, and many people, particularly young adults were in jeopardy [3]. Hypertension incidence was greatest in the country's Central, Western, and Eastern regions; demographic factors were not adequately accountable for the discrepancy in hypertension implication across the country [4]. Hypertension increases the risks to cardiac dysfunction, cerebral stroke, and kidney failure and affects other organs. It is a subtle menace to people's health all across the world [5].

Although the comprehension of hypertension's pathophysiology has progressed significant at the present, it remains extremely challenging to pinpoint the reasons and develop effective treatment techniques [6]. Approximately 80% of the global population, primarily in less developed regions, depend on natural remedies for their primary healthcare needs [7]. Natural products should be considered the finest in basic health care since they are more culturally acceptable, safer, more potent, less expensive, and have less side effects [8]. Several herbal medicines and supplements have been investigated as possible treatment agents for hypertension and associated complications. Given the high cost and frequently inaccessibility of conventional healthcare, the use of medicinal plants in basic healthcare is becoming increasingly popular in Uganda's rural areas. with about 70-80% of the Ugandans relying on medicinal plants for their medical treatment [9]. Plants and herbs such as the Adenopodia spicata, Agapanthus africanus, Agave Americana, Allium sativum, Amaranthus dubius, Amaranthus hybridus, Asystasia gangetica, Citrus limon, Citrus maxima, Clausena anisata, Crinum macowanii Baker, Dietes iridioides, and many other

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are used in hypertension treatment [10].

Citropsis articulate, the African cherry orange is a species of Rutaceae that can be found in various nations in West, Central, and East Africa [11], and it is a frequently used herb in disease management in Uganda [12]. The aphrodisiac characteristics are well-known among plant species with its leaf and root extract proving to increase testosterone levels hence it's used to manage erectile difficulties in males who have testosterone deficiency. In a recent research, its anti-plasmodial potential too was reported and it is popularly employed in management and treatment of malaria and erectile dysfunction in Uganda [13]. Citropsis articulata's antiplasmodial and aphrodisiac properties are thought to be attributable to phytochemical elements which includes: saponins, proteins, free amino acids, arginine, steroidglycosides, rutarins, coumarins, suberosin, seselin, di-methylsuberosin and haploperoside [14]. Given the various benefits of Citropsis articulate, it is believed that the plant also improves cardiovascular health [13].

Various studies that have been conducted show the effects of plant extracts to either significantly reduce or increase the mean arterial pressure of animals. In the study conducted by Gundamaraju et al. [15] indicated that Strophanthus hispidus decreased the mean arterial pressure during renal artery occlusion.

Sodium helps neurons and muscles especially the cardiac muscle to operate properly [16]. The normal reference range of sodium is 135-147 mmol/L, however different assays produce possible variations in reference ranges [17]. Serum sodium on the Architect c System, has a reference range of 136-145 mmol/L [18].

Various Studies have been done on a critical examination of the usage of plant-based seasonings to reduce salt levels in foods [19].

The standard serum potassium level falls within the range of 3.5 to 5.0 mEq/L in healthy people according to studies conducted by [20]. Many physiologic functions rely on the homeostatic maintenance of serum K+ [21]. Elevated potassium levels cause changes in the resting trans-membrane potential and affects the excitability of cardiac tissue reducing the action of the heart & blood pressure and improving cardiac function [22].

The body's most common ion is calcium, and its active form in the body is serum ionized calcium, which must be strictly regulated to prevent systemic toxicity, particularly of the nervous, cardiovascular, and urinary systems [22]. Serum calcium concentration exceeding 10.5 mg/dL (>2.5 mmol/L) is known as hypercalcemia, with considerable inter-laboratory variance in the reference range of 8-10 mg/dL (2-2.5 mmol/L) [23]. In plasma, calcium is found in protein-bound, complexed, and ionized forms to variable degrees [24]. About 45 percent of blood calcium is associated to albumin plasma protein predominantly, with the remainder bound to other proteins. Globulins bind to smaller levels. Calcium is bound to fibrin at a minimal proportion. As a result, Calcium levels in serum and plasma hardly differ from one another [25]. 45 percent more of the serum calcium is ionized [26]. Negatively charged ions like bicarbonate, phosphate, and sulfate make up the final ten percent of serum calcium [27].

Materials and Methods

Study location

The experiment was carried out at the physiology department laboratory, at Kampala International University-Western Campus.

Plant collection, identification and extraction

Citropsis articulata plant roots were obtained from the wild at Omukiyenje settlement, Masha Sub County, Isingiro district, Southwestern Uganda. A herbarium sample was developed by collecting roots, which was sent to Makerere University's College of Natural Sciences Herbarium for identification, where the plant was identified, and a voucher specimen number assigned. Citropsis articulata aqueous root extract was obtained through the use of a procedure previously documented by Gakunga et al. [28]. The roots underwent a thorough washing with tap water to eliminate any traces of dirt or soil, ensuring the use of fresh and clean water for this process. The cleaned roots of C. articulata were dried under a shade at room temperature for four weeks to prevent the loss of a volatile bioactive chemical. Using a mortar and pestle, the dried roots were ground into a fine powder. Approximately 1.5 kg of fine root powder was obtained, weighed using a balance and boiled in 10 liters of distilled water for 120 minutes at 70oC to extract the active components. It also mimics the traditional method of making the remedy for malaria and erectile difficulties treatment [29]. After cooling, filtration of the extract was done using cotton wool and later carefully filtered with Whatmann filter paper.

Afterward, the filtrate underwent concentration using a rotary evaporator at 50°C, and it was subsequently oven dried at 40°C until it achieved a semi-solid consistency. The extract that ensued was weighed and then placed in a refrigerator, and stored at 4°C. Extract's fresh aliquots were prepared daily using distilled water to achieve concentrations of (200, 400, and 600) mg/kg. Citropsis articulata was extracted by hot maceration by macerating 1000g of pulverized C.articulata root powder in 2000ml of distilled water. Citropsis articulata root powder used in the study yielded 72 g of the crude extract, which was then used in subsequent tests.

Determination of sample size

The quantity of animals to be employed in the study was established by the formulae n = DF/K+1 where; (n) is number of rats per group , DF is the degree of freedom, K is the number groups and the DF can either be 10 or 20 at maximum [30]. The overall number of rats to be used in the study (N) was calculated by the formulae; N= n*k

According to this study, 6 groups were used.

Since n=DF/K+1

At 10 degrees of freedom, n=10/6+1 n=1.4

At 20 degrees of freedom, n= 20/6+1 n= 2.8

The number of rats to be used in the study (N) = n*K

N = 1.4*6 N =8

N = 2.8*6 N =16

Experimental protocol

Twenty-four male Wistar rats, 4- 6 weeks old, weighing above 150g were procured from the Animal house at Kampala International University-Western Campus. The animals were confined to their cages, 4 Wistar rats per cage under a 12-hour cycle of light and dark. Acclimatized for a week during which time they were fed a regular pellet diet and given water ad libitum [31].

Induction of hypertension

Hypertension was induced in rats in group II, III, IV, V and VI of the six groups using 50 mg, 21-day release of Deoxycorticosterone

acetate salt for 42days which was administered subcutaneously once every 21 days and feeding the rats with 1% sodium chloride salt in the drinking water [32]. This model had a stifled manifestation of elevated aldosterone which resulted in raised sodium and water reabsorption from epithelial cells in the kidney's distal nephron, thereby elevating blood pressures [33].

Grouping of experimental animals

The 24 male Wistar rats were clustered in six groups each with four rats [n = 4]. Group I (non-hypertensive), the control group, were fed on the standard diet of pellet and provided with water ad libitum daily for 42 days. Group II (hypertensive) were injected with deoxycorticosterone acetate, fed on the standard pellet diet and 1% sodium chloride in the water ad libitum daily for 42 days. Group III (hypertensive) were injected with deoxycorticosterone acetate, fed on the standard pellet diet and 1% sodium chloride in the water ad libitum and treated with 200mg/kg of Citropsis articulata daily for 42 days. Group IV (hypertensive) were injected with deoxycorticosterone acetate, fed on the standard pellet diet and 1% sodium chloride in the water ad libitum and treated with 400mg/kg of Citropsis articulata daily for 42 days. Group V (hypertensive) were injected with Deoxycorticosterone acetate, fed on the standard pellet diet and 1% sodium chloride in the water ad libitum and treated with 600mg/kg of Citropsis articulata daily for 42 days. Group VI (hypertensive) were injected with Deoxycorticosterone acetate, fed on the standard pellet diet and 1% sodium chloride in the water ad libitum, and treated with a dose of 1mg/kg propranolol daily for 42 days (Table 1).

Extraction and storage of blood and tissues

Using aseptic techniques, each rat was handled by lifting it from its tail (caudal end) with the right hand, and then allowed to grip the wire mesh of the cage with its fore legs. The animal was then grasped from the nape of the neck between thumb and index finger. The tail was then transferred from the right to the left hand in between the small and ring fingers and carried using a one-handed method. Following handling, the rats were restrained. They were then anesthetized with 60 mg/kg of ketamine and 5mg/kg of xylazine intra-peritoneally and blood samples obtained by Cardiac puncture and collected in clot activating tubes [34].

Determination of blood pressure

Blood pressure was measured using a power lab non-invasive blood pressure machine. The equipment employed included a retainer featuring an inflatable tail cuff, a pressure transducer, a mercury sphygmomanometer, a photoplethysmograph (PPG), and a recording system known as BIOPAC MP 30. It was crafted using a thin (0.5mm) translucent rubber material, with dimensions measuring 1 cm in width and 3 cm in length. This was inserted into a cuff made of stiff fabric, wrapped around the tail, and fastened with velcro. The bladder was next connected to a mercury sphygmomanometer via a thin, non-collapsible rubber tube which after the systolic and diastolic pressure measured.

Determination of serum electrolytes (Na+, k+, ca2+)

Determination of serum sodium (Na+)

Serum sodium was measured using the Ion selective electrodes (ISE) method of measuring electrolytes. Sodium ISE comprises a unique glass which is sodium selective and a Reference electrode containing a calomel or silver chloride electrode. The data was interpreted using the following reference (normal) sodium ranges 135-147mmo/L.

Determination of serum potassium (k+)

Serum potassium was measured using the Ion selective electrodes method of measuring electrolytes. The antibiotic valinomycin was immobilized in potassium ISE, which is made of polyvinyl chloride (PVC). The potassium ions are specifically transported across the membrane by valinomycin and a Reference electrode containing a calomel or silver chloride electrode. The results were interpreted in accordance with the reference ranges.

Determination of serum calcium

Serum Calcium was measured using the Ion selective electrodes method of measuring electrolytes. The antibiotic valinomycin was immobilized in potassium ISE, which is made of polyvinyl chloride (PVC). The Calcium ions are specifically transported across the membrane by valinomycin and a Reference electrode containing a calomel or silver chloride electrode. The results were interpreted in accordance with the reference ranges.

Statistical analysis and data presentation

The data was analyzed using Graph pad prism version 6.0, San Diego California, USA. The data was presented as Mean ± SD and significance was assessed at p-value of less than 0.05. Statistical comparisons were conducted using one way ANOVA, followed by Turkey's post-hoc multiple comparison tests to identify significant differences across the groups.

Ethical considerations

Animals involved in the research were subjected to humane treatment, following the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" by the National Institute of Health [4]. The Uganda National Council for Science and Technology (UNCST) offered ethical research guidelines, which was followed during the research period. It was underlined that the number of rats employed in the research be minimized, plus careful disposal of research materials.

There was provision of ample space, adequate fresh air, cycles of 12-hour cycle of light and dark exposure, bedding materials that are comfortable and a high structure to keep rats safe from predators and secondary infection sources. Water and feeds were availed and Incineration used to get rid of deceased animals.

Table 1: Animal grouping.

Group (n=4)	Name	Treatment	Type of feed
I	Non-hypertensive	None	Standard diet
II	Hypertensive	None	21-day release, 50 mg of DOCA salt for 42 days
III	Hypertensive	*C. articulata* 200 mg/kg	21-day release, 50 mg of DOCA salt for 42 days
IV	Hypertensive	*C. articulata* 400 mg/kg	21-day release, 50 mg of DOCA salt for 42 days
V	Hypertensive	*C. articulata* 600 mg/kg	21-day release, 50 mg of DOCA salt for 42 days
VI	Hypertensive	1 mg/kg propranolol	21-day release, 50 mg of DOCA salt for 42 days

Results

Effects of C.articulata on blood pressure

The mean arterial pressure was calculated from formulae: DBP+1/3(SBP – DBP), where DBP = diastolic blood pressure & SBP = systolic blood pressure. The systolic pressure and diastolic pressures (mmHg) of standard feed group were 118.3 ± 7.68 and 81.5 ± 3.45 respectively with a mean arterial pressure of 93.7 ± 2.86. The systolic pressure and diastolic pressures (mmHg) of the DOCA salt group were 160.5 ± 113.7 and 109 ± 11.6 respectively with a mean arterial pressure of 126.2 ± 7.32. The systolic pressure and diastolic pressures (mmHg) of the DOCA salt + C.articulata 200mg/kg group were (150 ± 10.75) and 98.0 ± 3.65 respectively with a mean arterial pressure of 115.6 ± 5.9. The systolic pressure and diastolic pressures (mmHg) of the DOCA salt + C.articulata 400mg/kg group were 139.0 ± 10.52 and 89.25 ± 2.22 respectively with a mean arterial pressure of 105.8 ± 4.16. The systolic pressure and diastolic pressures (mmHg) of the DOCA salt + C.articulata 600mg/kg group were 132.0 ± 5.42 and 86.25 ± 6.45 respectively with a mean arterial pressure of (101.5 ± 5.21). The systolic pressure and diastolic pressures (mmHg) of the DOCA salt + Propranolol 1mg/kg group were 127.0 ± 2.45 and 82.5 ± 3.32 respectively with a mean arterial pressure of 97.34 ± 1.94.

The mean arterial pressure (mmHg) for the DOCA salt group (126.2 ± 7.32), DOCA salt + C.articulata 200mg/kg (115.6 ± 5.9), DOCA salt + C.articulata 400mg/kg 105.8 ± 4.16, DOCA salt + C.articulata 600mg/kg (101.5 ± 5.21) were significantly increased (P< 0.05) when compared to the standard feed group (93.7 ± 2.86). The mean arterial pressures (mmHg) for the DOCA salt + C.articulata 400mg/kg (105.8 ± 4.16) and DOCA salt + Propranolol 1mg/kg 97.34 ± 1.94 were significantly decreased (P< 0.05) when compared to the DOCA salt group (126.2 ± 7.32). The mean arterial pressures (mmHg) for the groups of DOCA salt + C.articulata 600mg/kg 101.5 ± 5.21, and DOCA salt + Propranolol 1mg/kg (97.34 ± 1.94) were significantly decreased (P< 0.05) when compared to the DOCA salt + C.articulata 200mg/kg (115.6 ± 5.9) group (Figure 1).

Figure 2: Effects of aqueous root extract of Citropsis articulata on a systolic blood pressure, diastolic blood pressure, and mean arterial pressure on DOCA salt- Induced hypertensive male wistar rats. n= 4, values expressed in mean ± SD.

KEY

* Significant to standard feed group

Significant to DOCA salt group

† Significant to DOCA + C. articulata at 200mg/kg

Effects of C. articulata on serum electrolytes (sodium, potassium, chloride)

Effects of C. articulata on sodium ion levels

The serum sodium ion levels (mmol/L) for the DOCA salt group (189.5 ± 26.1,) was significantly increased (p<0.05) when compared to the standard feed group (144.8 ± 6.81). The serum sodium ion levels in the DOCA salt + C. articulata 200mg/kg (152.3 ± 11.03), DOCA salt + C. articulata 400mg/kg (140.3 ± 9.54,), DOCA salt + C. articulata 600mg/kg (136.0 ± 8.83) and DOCA salt + Propranolol 1mg/kg (139.8 ± 7.32) groups were significantly decreased (p<0.05) when compared to the DOCA salt group (Figure 2).

Figure 3: Effects of aqueous root extract of Citropsis articulata on a

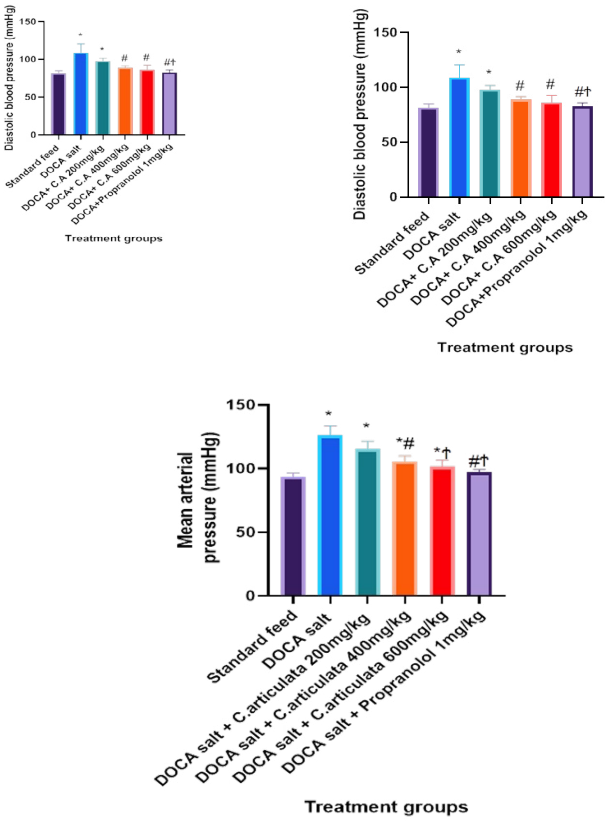

Figure 1: Effects of aqueous root extract of *Citropsis articulata* on a systolic blood pressure.

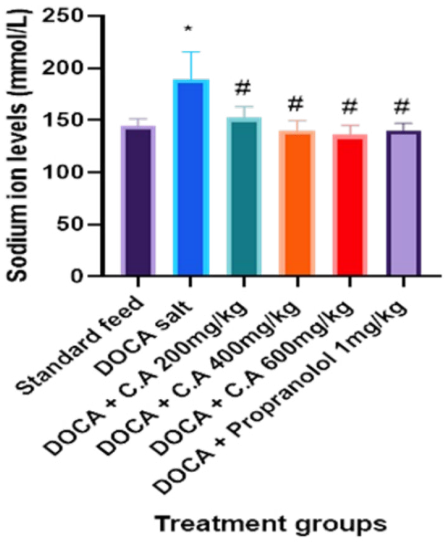

Figure 2: Effects of aqueous root extract of *Citropsis articulata* on a serum sodium levels on DOCA salt.

Serum sodium levels on DOCA salt- Induced hypertensive male wistar rats. n= 4, values expressed in mean ± SD.

KEY

* Significant to standard feed group

Significant to DOCA salt group

Effects of C.articulata on potassium ion levels

There was no significant decrease or increase in the serum Potassium levels (mEq/L) in the groups of DOCA salt (5.75 ± 1.53), DOCA salt + C. articulata 200mg/kg (5.15 ± 1.70), DOCA salt + C. articulata 400mg/kg (4.83 ± 0.70), DOCA salt + C. articulata 600mg/kg (4.73 ± 0.85) & DOCA salt + Propranolol 1mg/kg3.93 ± 0.36 mEq/L when compared to the standard feed group (3.83 ± 1.48) mEq/L (Figure 3).

Figure 4: Effects of aqueous root extract of Citropsis articulata on a Serum Potassium levels on DOCA salt-Induced hypertensive male wistar rats. n= 4, values expressed in mean ± SD.

Effects of C. rticulata on calcium levels

There was no significant increase or decrease of the serum calcium levels (mmol/L) in the groups of DOCA salt (2.20 ± 0.93), DOCA salt + C. articulata 200mg/kg (2.33 ± 0.71), DOCA salt + C. articulata 400mg/kg (2.50 ± 0.54), DOCA salt + C. articulata 600mg/kg (3.2 ± 0.54) and DOCA salt + Propranolol 1mg/kg (3.08 ± 0.77 mmol/L) when compared to the standard feed group (3.13 ± 1.08) (Figure 4).

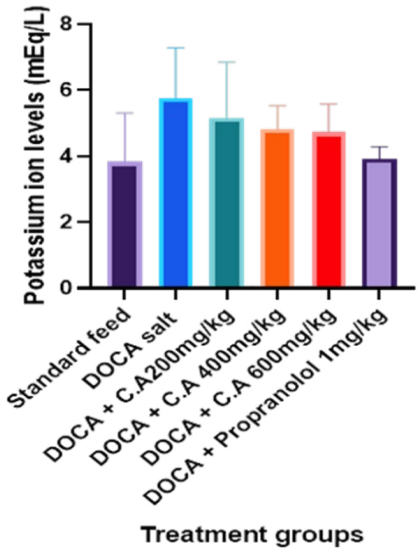

Figure 3: Effects of aqueous root extract of *Citropsis articulata* on a serum potassium levels on DOCA salt.

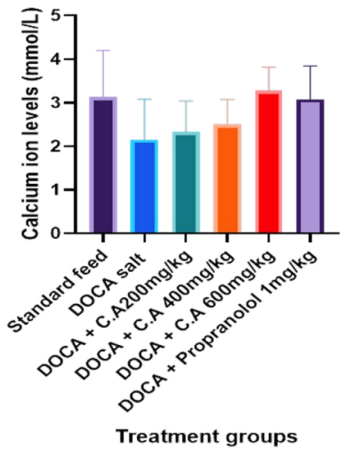

Figure 4: Effects of aqueous root extract of *Citropsis articulata* on a serum calcium levels on DOCA salt.

Effects of aqueous root extract of Citropsis articulata on a Serum Calcium levels on DOCA salt- Induced hypertensive male wistar rats. n= 4, values expressed in mean ± SD.

Discussion

The study examined how the effects of aqueous root extract of C.articulata on blood pressure DOCA salt-Induced hypertensive male wistar rats put into six groups, each with 4 animals. Hypertension was induced using Deoxycorticosterone Acetate 50mg for 42 days in a 21-day release subcutaneously. On the 42nd day, the rats were euthanized and serum electrolytes assessed.

DOCA salt belongs to mineralocorticoid ester a group of mineralocorticoids and widely used in the management of hypo-adrenocorticism. However, with a major effect in causing hypertension [35]. Thus, a therapeutic approach to prevent DOCA salt induced hypertension could have a positive impact [36].

The mean arterial pressure (MAP denotes the average arterial pressure over an entire cardiac cycle, encompassing both systole and diastole. MAP is affected by cardiac output and systemic vascular resistance, both of which are influenced by multiple factors, and can be calculated using the formula MAP = DP + 1/3(SP – DP), where MAP = mean arterial pressure, SP = systolic pressure & DP = diastolic pressure. The study showed that DOCA salt administration significantly increased the mean arterial pressure which was similar with a past study done by [37] which elaborated that animals which were fed with DOCA salt exhibited and elevated MAP. The elevation in mean arterial pressure could have resulted from changes in the control of central presser mechanisms, which include the brain renin-angiotensin system, the cardiovascular system, and baroreflex responses [38]. High mean arterial pressure contributes to increased oxygen demand by the heart, ventricular hypertrophy, and end organ damage which elaborated the undesirable effects [39]. However, the administration of aqueous root extract of C. Articulata markedly decreased the mean arterial pressure which elaborated the ameliorative effects of the plant and showed similarities with studies conducted by Anastasiou & Buchbauer [40]. Additionally, it was observed that propranolol treatment significantly reduced the mean arterial pressure in the DOCA salt induced wistar rats which showed similarities with previous studies done by Foshati et al. [41].

Electrolytes are minerals that produce an electric charge when they disperse in fluids. The body produces electrolytes, and they are also obtained from dietary sources, beverages, and supplements. Electrolytes in blood, tissues, and urine are important for maintaining fluid balance, controlling heart rhythm, and facilitating nerve and muscle function [42]. The DOCA salt administration causes cardiac function impairment by sodium retention which in turn increases the blood pressure levels by retaining water, increasing systemic peripheral resistance and altering endothelial function [43]. The study elaborated a significant increase in serum sodium ion level in the DOCA salt induced groups, however, showed no significant changes in the serum potassium and calcium ion levels following DOCA salt administration which shows similarities with previous studies done by [44] but contradicted studies conducted by Cardoso et al. [45]. Administration of aqueous root extract of C. articulata showed significant decrease in the serum sodium ion levels but with no significant changes in the levels of Potassium or calcium. The observed decrease in serum sodium ion with no changes in the serum Potassium and Calcium showed similarities with studies done by Koriem et al. [46]. It was also observed that propranolol treatment in the DOCA induced groups significantly

reduced serum sodium but showed no effect on the Potassium and Calcium levels which illustrated similarities with studies done by Wei et al. [47]; Kolkhof & Barfacker [48].

Conclusion

In conclusion, the study demonstrated that Deoxycorticosterone Acetate salt (DOCA salt) administration induced hypertension in male Wistar rats.

Citropsis articulata was seen from the study, to have potential in improving blood pressure due to bioactivity of its phytochemical components.

Recommendations

It is recommended that this study should also be carried out in female rats to ascertain the level of DOCA salt that can cause hypertension and the possible effects of Citropsis articulata.

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