Effect of Viscum album (mistletoe) extract on some serum electrolytes, organ weight and cytoarchitecture of the heart, kidney and blood vessels in high salt fed rats

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Citation

Abstract
The effects of Viscum album (mistletoe) on some serum electrolytes, organ weight and cytoarchitecture of the heart, kidney and descending aorta were carried out in this study. 24 male albino Wistar rats (150g and 200g final body weight) were divided into 4 groups of 6 rats each and were fed on either normal rat pellet, high salt (8% NaCl) diet and/or treated with mistletoe extract for 6 weeks. Salt fed (untreated) group (group 3) lost body weight of 1.13% at week 6, while other groups gained weight, they had reduced mean heart and kidney weights relative to other groups. Na$^+$, and Cl$^-$ concentrations in group 3 were significantly (P<0.01) higher, while K$^+$, HCO$_3^-$ and Ca$^{2+}$ levels in group 3 were lower than in other groups. Photomicrograph of the group 3 rats revealed excoriated vascular endothelium and thickening of the tunica media, while the extract fed group had almost similar cytoarchitecture as the control group. Histology of the heart of group 3 showed hypertrophied myofibrils and myoplasm with numerous nuclei with damaged and sparse renal interstitium. In conclusion, high salt load increases serum Na$^+$ and Cl$^-$, and decreases K$^+$, HCO$_3^-$, and Ca$^{2+}$. It also causes reduction in body, heart and kidney weights and damage to the tissues of the heart, kidney and blood vessels. These adverse effects of salt load were ameliorated following chronic feeding on mistletoe extract.

INTRODUCTION
The history of the use of herbs dates back to the time of the early man. Among herbal plants used in treatment of ailments is mistletoe (Viscum album), an idiosyncratic plant surrounded by age-old traditions of ancient cult and magic rites. It is an evergreen semi-parasitic plant that grows primarily on deciduous trees. It is widely distributed throughout Europe, North Africa, Austria, Asia and also in Nigeria. Phytochemical screening of Tapinanthus dodoneifolius (DC) Danser called “Kauchi” in Hausa a species of African mistletoe, showed the presence of anthraquinones, saponins, tanins. European mistletoe has gained widespread research. The primary chemical constituents have been found to vary according to the host plant but typically include glycoprotein, polypeptides (Viscotoxin), flavonoids, flavonol agylcones, lectins, triterpenes, saponins, caffeic acid, lignans, choline derivatives related to acetylcholine, vitamin C, histamine, resins, thionins, cardionolids and phenolic compounds. A decoction of the leaves of mistletoe is traditionally used in the treatment of a number of ailments, including hypertension. It has been observed that methods used by traditionalist in early diagnosis lack scientific basis.

High salt intake in humans is associated with numerous complications; it can elevate blood pressure in some individuals. In experimental animals it causes endothelial dysfunction, increase in plasma brain natriuretic peptide concentration and perivascular inflammation, deactivation of ATP-sensitive potassium channels and Na-k ATPase pumps on the vascular smooth muscle membrane etc. With paucity in literature on the effect of mistletoe extract on serum electrolytes and cytoarchitecture of the kidney, blood vessels and heart following high salt intake, it is therefore the aim of the present study to scientifically investigate the effects of chronic consumption of mistletoe extract on some serum electrolytes and cytoarchitecture of the heart, kidney and blood vessels in rats.

MATERIALS AND METHODS
Effect of Viscum album (mistletoe) extract on some serum electrolytes, organ weight and cytoarchitecture of the heart, kidney and blood vessels in high salt fed rats

EXPERIMENTAL ANIMALS
24 growing male albino Wistar rats were obtained from the animal house of the Department of Medical Physiology, University of Calabar, Nigeria. The rats were initially weighing between 70g and 90g but their final weight was between 150g and 200g when they were used for the experiment. The rats were weighed before commencement of the feeding experiment and thereafter were weighed daily. They were nursed under controlled environmental condition.

EXPERIMENTAL PLANT
2kg of fresh leaves of mistletoe were purchased from a local plantation in Akpabuyo Local Government Area of Cross River State, Nigeria during the rainy season and were identified as Viscum album by a botanist (Mr. Frank Adepoju) in the Department of Biological Sciences, University of Calabar, Nigeria.

PREPARATION OF PLANT EXTRACT
Fresh leaves of Viscum album from the host plant (citrus) were collected. The leaves were first washed free of sand and debris. Wash water was blotted off and the leaves ground to paste. A quantity of the ground sample (50g) was weighed and soxhlet extracted with 150ml distilled water at 100°C for 9hr. Where larger ground samples were used, extraction was done under reflux with an appropriate volume of distilled water. The extract was slowly evaporated to dryness in vacuo at 40°C using a rotary evaporator. A total yield of 31% was obtained. Weighed samples of the extract were then used to prepare the stock solution after the method of Eno et al., 19.

PREPARATION OF HIGH SALT DIET
High salt diet containing 8% sodium chloride was prepared using a standard diet containing 0.3% sodium chloride after the method of Obiefuna et al., 19 and Akinyanjuola, 22.

ACUTE TOXICITY TEST
Seventy male white mice (18-20g) were used for the study. They were randomly selected and assigned to 7 cages of 10 animals per cage. They were allowed a week for adaptation. Each group then received one of the following doses: 0, 125, 250, 500, 1000, 2000 and 4000 mg/kg body weight of the extract, i.p. The maximum volume injected was 0.2mls. The groups were then returned to the home cages and allowed free access to food and drinking water. The mortality in each cage was assessed 24 hours after the administration of the extract. Percentage mortalities were converted to probits and plotted against the log_{10} of the dose of the extract from which the LD_{50} value was extrapolated following the method of Miller and Tainter 22.

EXPERIMENTAL PROTOCOL
The twenty-four male albino Wistar rats were divided into 4 groups of 6 rats each. They were fed as follows: Group 1 (control) was fed on normal rat pellet + drinking water. Group 2 was fed on normal rat pellet + drinking water + 150mg/kg of mistletoe extract orally once daily. Group 3 was placed on high salt diet (8% sodium chloride) + 1% sodium chloride drinking water. The group 4 received same as the third group + mistletoe extract (150mg/kg body weight) orally once daily. The feeding regimens lasted for six weeks. At the end of the feeding period, the animals were sacrificed and blood sample collected for daily analysis. The animals were weighed daily.

COLLECTION OF BLOOD SAMPLES
The animals were made unconscious using chloroform anaesthesia and blood collected via cardiac puncture (blood was drawn from the heart) a modification of the method by Ohwada 23. The samples were collected by the help of 5mls syringe attached to needle (21 SWG) into plain capped bottles. The samples were immediately used for the estimation of the different variables.

COLLECTION AND WEIGHING OF ORGANS
The rats were sacrificed under chloroform anaesthesia. They were quickly dissected and their kidney, heart and blood vessels were removed and weighed using the mettler P163 weighing balance.

HISTOLOGY OF THE LIVER AND KIDNEY
Permanent preparations using routine biopsy method was employed. Tissue sections were treated with traditional haematoxylin and eosin stains. The tissue blocks from the heart, kidney and blood vessels were fixed in 10% neutral formalin after which they were dehydrated using alcohol and then cleaned in xylene. They were then embedded in paraffin wax and thin sections cut at 5 microns. The sections were then stained with haematoxylin for 15 minutes, differentiated with 1% acid alcohol, counter stained in eosin for 2 mins and mounted with DPX. The sections were then viewed under the microscope (x400) and photomicrograph taken.

MEASUREMENT OF SERUM ELECTROLYTES
Blood samples from each rat was collected separately into
clean capped plain tubes and allowed to stand for 30 minutes for clotting to occur. These were then centrifuged at 2500 revolution per minute for 15 minutes. The serum was extracted into clean test tube for the analysis of sodium, potassium, chloride, bicarbonate and calcium ions.

**MEASUREMENT OF SERUM ELECTROLYTES**

Sodium and potassium ions were measured using Flame photometry method at wave length of – 590nm for sodium and 770nm for potassium.

Bicarbonate ions were estimated by titration method. Calcium Ions were measured by photometric test using O-cresophethalein – complexene in an alkaline medium.

Measurement of chloride ion was done by titration, using mercuric nitrate method of Schales and Schales.

**STATISTICAL ANALYSIS**

Data were presented as mean ± SEM. Data were analysed using a one way analysis of variance (Anova) then followed with post hoc test (Least Square Deviation). P value of less than 0.05 was declared as significant statistically.

**RESULT**

**ACUTE TOXICITY TEST**

The LD$_{50}$ value for the crude extract from V. album leaves was extrapolated to be 420.70mg/kg mice i.p ($r = 0.9515$; $P<0.01$, $n = 10$) from which a test (safe) dose of 150mg/kg body weight was derived for the chronic feeding experiment.

**BODY WEIGHT**

Figure 1 show mean body weight in control and test groups. In the control group, the initial (day 1) mean body weight was 84.00±6.26g. It rose to 174.17±14.76g on the last day (107.35%). This was equivalent to a growth rate of 2.15g per day. Animals in group 2 (normal + extract) had an initial body weight of 93.00 ± 2.25g and 123.17±5.17g on day 42 producing 30.17g (32.44%) change in body weight (growth rate of 0.72g/day). This shows a relative reduction in weight and growth rate in group 2 when compared with control rats.

The third group of animals (salt fed) had a loss in weight of 1.33g (1.13%) on the last day of the experiment (from an initial mean body weight of 118.00 ± 6.63g to 116.67± 9.03g final weight). Their growth rate was -0.03g/day. Rats in group 4 (salt fed + extract) had growth rate of 0.89g/day or total weight gain of 37.17g (39.77%). Their mean weight on the last day was 130.17±10.79g from an initial value of 93.00±2.25g.

**ORGAN WEIGHT**

In the control group, the mean weight of the left kidney was 0.58±0.11g. It was 0.53±0.03g, in group 2 rats, showing no significant difference from the control value. The salt fed group had a reduction in the mean weight of the left kidney of 0.45±0.03g, their mean weight was reduced when compared to the control; this was also not significantly different. But in the salt fed + extract group, there was a significant ($P<0.05$) increase in the mean weight of the kidney (0.53±0.03g) when compared to salt fed group. (Fig. 2).
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**Figure 2**

Figure 2: Comparison of weights of the heart and left kidney in control and test groups. Values are mean ± SEM. n = 6.

* = P

The mean heart weights in the control group were 0.61±0.02g (with mean body weight of 247.7±8.16g). The mean heart weight, 0.55±0.03g (having a mean body weight of 189.96±7.24g) in the salt fed group was significantly (P<0.01) lower compared with control value. The elevation in mean heart weight of group 2 (0.66±0.03g, with 181.37±7.66g as mean body weight) and group 4 (0.64±0.02g (with mean body weight of 190.2±5.45g) were not significant compared with control value, (Fig. 2).

**SERUM ELECTROLYTES**

Table 2 shows comparison of serum electrolyte concentration in the experimental groups. The mean value of sodium ion in control group was 142.17±0.65Meq/L; group 2 had slight reduction in sodium ion concentration (141.67±0.21Meq/L) compared to control. Salt fed (untreated) group had a significant increase in sodium ion concentration (144.33±0.42Meq/L) compared with normal control group. Group 4 (salt fed + extract) had a significant reduction in sodium level (139.5±0.67Meq/L) compared with control (P<0.05) and salt fed rats (P<0.001).

The mean value of potassium ion in control group was 4.18±0.07Meq/L. The mean potassium level in group 2 (4.60±0.04Meq/L) was significantly (P<0.001) higher than control value. The salt fed group had a potassium ion concentration of 3.96±0.42mEq/L which was significantly lower than that of the control group (P<0.05). Potassium ion concentration was significantly (P<0.001) reduced in the salt + extract group (5.03±0.05mEq/L) compared with both control and salt fed groups.

The mean chloride ion concentration of the normal control group was 103.00±0.44mEq/L. Normal + extract (group 2) had a mean chloride ion concentration of 101.33±0.42 mEq/L, which was significantly lower than that of the control group (P<0.05). The mean chloride ion concentration of the salt fed group (105.00±0.44mEq/L) was significantly higher (P<0.01) than that of the control group. The salt fed + extract group had a mean chloride ion concentration of 102.66±0.84mEq/L was not significantly lower than that of the control group but was significantly lower than that of the salt fed group (P<0.01).

The mean bicarbonate ion concentration of the normal + extract group was 24.00±0.36 mEq/L which was not significantly different from the normal control group. The salt fed group had also a slightly higher bicarbonate ion concentration (24.66±0.21mEq/L) which was not significantly different from the control group. However, the salt fed + extract group had a bicarbonate ion concentration of 26.66±0.42 mEq/L which was significantly different from both that of the control group and salt fed group (P<0.001 and P<0.01, n = 6 respectively). See Table 1.

HISTOLOGICAL STUDY

Plate 1a shows the photomicrograph of the longitudinal section (x 400) of the cardiac muscle in control group. It is characterized by cross striation and inter-digitations of the myofibrils with well stained myoplasm and nuclei and showing an outline of the normal heart. In the extract fed group the myocardium had blood stains and myofilbrils can not be properly distinguished as individual cells compared with control although, the nuclei are well stained, (Plate 1b). In high salt fed (untreated) group, the cardiac muscles had thicker muscular wall, showing alignment of the myofibrils with numerous nuclei indicating hyperplasia of the nuclei, the myofibrils were fused together, (Plate 1c). Also seen are
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the cross striations observed in the control plate. Plate 1d shows that in the high salt + extract fed group, the cardiac muscle had cross striation similar to control but with slightly thicker muscular wall than control.

**Figure 3**
Plate 1: Photomicrograph of the section of the heart in (a) control, (b) normal + extract fed, (c) salt fed and (d) salt + extract fed groups stained with H & E (x 400) showing the cardiac muscles.

Plate 2a shows the photomicrograph of the section of a descending aorta in normal control revealing the three basic layers of the blood vessel, the tunica intima (TI) with distinct epithelial lining resting on the basement membrane, tunica media (TM) of prominent stained nuclei and smooth muscles interlocked with elastic membrane. The tunica adventitia (TA) is also seen as a thin connective tissue layer which is also present with collagen and elastic fibers. In the group 2 animals, the three basic layers are intact with epithelial lining of the tunica intima, the tunica media has fewer stained nuclei with intact elastic tissues, and the tunica adventitia is less prominent than in control plate (Plate 2b). The high salt fed group also shows the three basic layers of the blood vessel but with slight erosion of the epithelial lining, the TM is thicker compared to the control plate but with fewer stained nuclei comparatively. TA is also less predominant, (Plate 2c). Plate 2d shows a photomicrograph of the section of the descending aorta in high salt fed plus extract group stained with H and E (x 400) showing the three basic layers with similar architecture as control.

**Figure 4**
Plate 2: Photomicrograph of the section of the descending aorta in (a) control, (b) normal + extract fed, (c) salt fed and (d) salt + extract fed groups stained with H & E (x 400) showing the cardiac muscles.

As shown in Plate 3a, the section of the kidney in control group shows segments of the renal cortex and intact glomerulus, which are normally placed; the nuclei of the glomeruli are well distinguished. In the normal + extract fed group (Plate 3b) the glomerulus and renal cortex are obliterated, creating a wider renal bowman space for filtration. Although, the nuclei of the cells and juxtaglomerular capsules were not distinctly shown, rather shows well stained cells of the glomerular apparatus which were also very well outlined. Tubular vessels are also well outlined. The section of the kidney in salt fed group is shown in Plate 3c, characterized by obliteration of some segments of the glomerulus, with sparse interstitium, creating greater renal space for excretion. This section also shows enormous tubular necrosis with massive degeneration of the cells of the glomerular apparatus (atrophic changes) showing yellow pigments, this plate has low staining intensity, and the renal vessels are not well outlined. Plate 3d shows the section of the kidney of group 4, showing segments of the renal cortex having well outlined glomerular tuft, creating a small renal space compared to plate 3c. The Juxtaglomerular apparatus is not clearly outlined and the
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tubules cannot be clearly identified and nuclei not well stained. Blood vessels are not properly distinguished and slight necrosis is also present.

Figure 5
Plate 3: Photomicrograph of the section of the kidney in (a) control, (b) normal + extract fed, (c) salt fed and (d) salt + extract fed groups stained with H & E (x 400) showing the cardiac muscles.

Figure 6
Table 1: Comparison of mean values of serum electrolytes in control and test groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Na⁺ (mEq/L)</th>
<th>Cl⁻ (mEq/L)</th>
<th>K⁺ (mEq/L)</th>
<th>HCO₃⁻ (mEq/L)</th>
<th>Ca²⁺ (mMol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>142</td>
<td>103.00</td>
<td>4.18</td>
<td>24.00</td>
<td>3.20</td>
</tr>
<tr>
<td>± 0.06</td>
<td>± 0.45</td>
<td>± 0.07</td>
<td>± 0.37</td>
<td>± 0.11</td>
<td></td>
</tr>
<tr>
<td>Group 2 (Normal + extract)</td>
<td>141.67</td>
<td>101.33</td>
<td>4.80</td>
<td>25.00</td>
<td>3.23</td>
</tr>
<tr>
<td>± 0.21</td>
<td>± 0.42*</td>
<td>± 0.04***</td>
<td>± 0.36</td>
<td>± 0.04</td>
<td></td>
</tr>
<tr>
<td>Group 3 (Salt fed)</td>
<td>144.33</td>
<td>105.00</td>
<td>3.97</td>
<td>24.67</td>
<td>3.00</td>
</tr>
<tr>
<td>± 0.42*</td>
<td>± 0.45**</td>
<td>± 0.04*</td>
<td>± 0.21</td>
<td>± 0.03</td>
<td></td>
</tr>
<tr>
<td>Group 4 (Salt + extract)</td>
<td>135.50</td>
<td>102.67</td>
<td>5.03</td>
<td>26.67</td>
<td>3.27</td>
</tr>
<tr>
<td>± 0.67***</td>
<td>± 0.84*</td>
<td>± 0.06***</td>
<td>± 0.42***</td>
<td>± 0.11***</td>
<td></td>
</tr>
</tbody>
</table>

* = P<0.05, ** = P<0.01, *** = P<0.001 vs control.
α = P<0.05 vs group 3; β = P<0.01 vs group 3; δ = P<0.001 vs group 3

DISCUSSION
The effects of high salt loading and treatment with crude extract of Viscum album (mistletoe) on serum electrolyte, organ weight and cytoarchitecture of the heart, kidney and blood vessel were carried out in this study on Wistar rats. It was observed that sodium ion concentration of high salt loaded rats was significantly higher than in the control rats. The increase in sodium ion concentration in the salt loaded rats is quite consistent with earlier reports that salt loading leads to elevated plasma levels of sodium ion [26, 27]. Salt is not readily excreted from the body when ingested. Rather, it indirectly increases the extracellular fluid volume via two basic mechanisms, it increases the osmolarity of the body fluids which stimulate the thirst center causing increased water intake and also stimulates the hypothalamic posterior pituitary gland secretion mechanism to secrete increase quantities of anti diuretic hormone [26]. It was also observe that there was a reduction in the sodium ion level in the extract fed group compared with the control and salt loaded rats. It is possible that the extract acts to increase the kidney excretion of this ion by a mechanism that remains to be investigated.

It was also observed that the extract fed group had increased potassium ion levels compared to the control and salt loaded rats, while the salt loaded rats had significantly reduced potassium ion levels. It is already known that sodium ion and potassium ion work and operate inversely as a result of the activity of sodium potassium pump [28, 29]. Increased excretion of sodium ion by the extract would have resulted in a concomitant reabsorption of potassium ion from the renal tubules.

The increased chloride ion concentration observed in the salt loaded rats is not surprising, since they were fed on high sodium chloride diets. Increased sodium retention is associated and directly related with chloride ion, since most sodium ion reabsorption is coupled with chloride ion reabsorption [30]. Extract fed groups were found to have reduced chloride ion levels; probably due to the reduced plasma levels of sodium ion in these groups of rats as explained in previous study. The excretion of chloride ion would naturally follow the excretion of sodium ions [27].

The extract fed rats were also observed to have elevated amount of bicarbonate ion levels. Chloride ion reabsorption is associated with excretion of bicarbonate ion; as the plasma chloride ions decrease, the bicarbonate ions increases to keep the total concentration constant [27,30]. This principle explains the action of the extract in maintaining homeostasis by causing reabsorption of bicarbonate ion as the plasma chloride ion reduces.
The extract fed groups were observed to have increased concentration of calcium ions. It has earlier been documented that the extract is rich in calcium ion. This could be the reason for the increase in calcium ion observed in the extract fed group compared to the control. Salt loaded rats in this work were also observed to have increase calcium level. This agrees with previous report that salt loading leads to excretion of calcium ions which probably could cause osteoporosis.

The salt loaded rats were observed to have reduced body weight and the least weight gain compared to other groups although they had the highest body weight at the start of the experiment. This reduction in body weight in the salt loaded rats would have been as a malabsorption due to consequence of intestinal damage caused by the high salt feed to the salt loaded rats. This will result in poor absorption of nutrients necessary for growth resulting in the lower weight recorded in the salt loaded group. Another probable reason for the lower weight of the salt loaded group may be as a consequence of some tissue damage and wasting caused by the high salt concentration. This finding is in agreement with previous reports which showed that salt loading caused derangement of several tissues, notably the lungs, liver and kidney. Salt loading causes insulin resistance and reduction in glucose uptake by the glucose receptors leading to loss in weight due to poor uptake of glucose by the tissues. Coelho et al., had reported that high salt diet caused decreased food intake accompanied with slow growth rate and decreased body weight.

The weight gain in the groups treated with the extract may be due to increase in plasma calcium concentration which causes calcium deposition in bones leading to increased bone density since an earlier documentation shows that the extract is rich in calcium. It is also possible that the extract would have prevented tissue and intestinal damage to enhance reabsorption of nutrient. It has also been reported that the extract has a blood glucose reducing effect which implies that the extract enhances glucose uptake by the tissues which could be probable reason for the better weight observed in extract fed group compared to the salt loaded rats. Cytoarchitecture of the salt fed + extract groups shows well outlined glomerular tuft and a small renal space compared to the salt fed group. This may be an effect of mistletoe extract that also reduced the tubular necrosis.

This study on organ weight shows decrease in mean weight of left kidney of the salt fed group. Histology of the renal cortex in this group of rats shows obliterations of some of the glomerulus, with sparse interstitium, greater renal space and enormous tubular necrosis with massive degeneration of cells (atrophic changes) of the juxtaglomerula apparatus. According to Boero et al., salt feeding causes renal morphological changes and progression of chronic renal failure. Earlier studies also show that high salt has detrimental effects on glomerular hemodynamics, inducing hyper-filtration, increased filtration fraction and glomerular pressure which also influence urinary excretion of protein.

The reduction in heart weight observed in the high salt loaded animals could be due to plasmolysis of cells, and poor feeding. It was also observed that the high salt loading led to increase in diameter of the tunica media of the descending aorta and cause slight erosion of the epithelial lining, when compared to the control. It is documented that salt loading causes endothelial damage. The mistletoe extract fed group was observed have similar architecture to the control indicating a healing potential of the extract.

**CONCLUSION**

In conclusion, high salt loading leads to increased levels of sodium and chloride ions which are implicated in the etiology of high blood pressure. It decreases the levels of potassium, bicarbonate and calcium ions and reduces body, heart and kidney weights which could predispose the body to cardiovascular diseases, osteoporosis etc., It also damages the heart, kidney and blood vessels. However, treatment with extract from Viscum album lead to a reversal of these adverse effects caused by salt loading and subsequently leads to improved on body weight.

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