Comparative Effect of Lisinopril and Amlodipine with Moringa Oleifera on the Kidney Function of Dexamethasone and Salt-Induced Hypertensive Albino Rats

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Abstract
Hypertension is one of the most common causes of death worldwide; and a common cause of cardiovascular and renal complications which can be frequent, chronic and age related. Use of antihypertensive drugs such as Lisinopril and Amlodipine which have no significant first pass effects, pose a threat to the kidney. This study aimed at comparing the effects of conventional antihypertensive drugs Lisinopril and Amlodipine with *Moringa oleifera* on the kidney of Albino rats using Urea and Creatinine as markers. A total of 30 Albino rats were divided into six groups. Group I served as negative control; Group II as positive control; Groups III, IV, V and VI were test groups. They were induced hypertension with Dexamethasone-salt and treated with 0.07mg/kg body weight Lisinopril, 0.27mg/kg Amlodipine and with 20mg/kg and 40mg/kg *Moringa oleifera* leaves capsules respectively. At the end of treatment which lasted for 3 weeks, the animals were sacrificed and blood samples collected into clean-dry containers and serum harvested; and used to measure the levels of Urea and Creatinine with Chemistry auto-analyzer (COBAS C111). Data obtained were analysed using statistical package for the social sciences (IBM-SPSS) version 27.0. The results indicated no significant increase in serum Urea and insignificant decrease in Creatinine in all the treatment groups. This indicates synergy in these classes of antihypertensive drugs and Moringa at the said concentrations. Based on these results, it can be concluded that *Moringa oleifera* leaves extract could be used complementarily with Lisinopril and Amlodipine for treatment and management of hypertension.

Introduction
Hypertension or elevated blood pressure is the force exerted by circulating blood against the arterial walls. It is a medical condition that increases the risk of heart, brain, kidney among other diseases [1]. It is a condition that often causes cardiovascular and renal complications that can be frequent, chronic and age related. It is one of the most common causes of death worldwide [2]. Cardiovascular disease has remained the leading cause of mortality in developed countries annually [3]. Inactivity, obesity and high sodium diet are some of the most predisposing factors to hypertension [4]. Most people with hypertension are not aware because in most cases there are no warning signs; however, when these symptoms occur, they include early morning headache, nosebleeds, irregular heart rhythms, changes in vision and buzzing in the ears. In severe cases, fatigue, nausea, vomiting, confusion, anxiety, chest pain and muscle tremors are experienced by the individual [5].

Lisinopril belongs to the class of antihypertensive drugs known as angiotensin-converting enzyme (ACE) inhibitors that are used majorly for the treatment of hypertension, heart failure, and after myocardial infarction. It is used in treating adults and children not less than 6 years old [6]. The major property that distinguishes it from other angiotensin-converting enzyme inhibitors is that it is hydrophilic, has a long half-life, increased tissue penetration and is not metabolized by the liver [7]. ACE inhibitors aid in dilating the blood vessels and thus reducing the blood pressure and lowering heart work load. Lisinopril is a first line of treatment in hypertensive patients [8]. It is taken orally either with or without food once daily, but

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countries recognize traditional medicines for their primary health care needs depending directly on medicinal plants for their medical purposes which is due to a number of reasons such as low cost, accessibility and affordability [14]. Moringa oleifera is commonly known as the miracle tree, horseradish-tree, or Ben oil tree. In the species of moringaceae, it is the best known and most widely distributed with a wide range of medical uses with high nutritional value worldwide. It is commonly found in India, Asia and Africa [15]. In Nigerian local languages, it is called Barambo or Zogale gandi in Hausa, Odudu Oyibo in Igbo and Ewele in Yoruba. This plant is used by different ethnic groups in Nigeria from the leaf, seed, flower, stem and root barks as food, tea and medicine; other parts are used in fencing and fuel for cooking [16]. Creatinine is the end product of creatine metabolism. It is the product of creatine phosphate breakdown in muscles, and is normally produced at almost a constant rate by the body which is dependent on the muscle mass of the individual. Blood creatinine also serves as a kidney marker because it is excreted unchanged by the kidney [17]. Creatinine is chiefly removed from the blood by the kidney through the glomerulus (glomerular filtration) and by proximal tubular secretion. There is increase in blood creatinine if there is deficiency in glomerular filtration or absence of tubular filtration. In this case, serum and urine creatinine can be used to calculate the creatinine clearance (CrCl), this approximately correlates with the glomerular filtration rate (GFR) [18].

Urea (diamide of carbonic acid) is the end product of nitrogenous and protein metabolism and is predominantly found in urine [19]. It is also a major end product of nitrogen metabolism in humans which is eliminated mainly by the kidneys in urine and it can also be secreted in the other body fluids such as blood, saliva etc. Urea drastically increases in pathological conditions and fed with standard diet and water ad libitum.

**Materials and Methods**

**Experimental Animals**

A total of thirty (30) male Albino Wistar rats weighing between 109g to 200g were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria. The animals were maintained at the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. They were allowed to acclimatize for one week under standard laboratory conditions in cages under normal environmental conditions and fed with standard diet and water ad libitum.

**Experimental Design**

Thirty male rats were randomly divided into six groups consisting of 5 animals each. The experiment consisted of two (2) basic periods, the inducement period and the treatment period. The former lasted for five days, during which hypertension was induced in animals in groups II - VI using Dexamethasone and 4% sodium chloride based on individual body weight. The animals were given Dexamethasone once and sodium chloride solution daily, all through the inducement period. Animals in certain groups were treated with Lisibopril, Amlodipine and Moringa Oleifera (with dosage varying with different groups). Three (3) animals from groups III, IV and VI were sacrificed at the end of each treatment week, along with those of the control groups I and II; and samples analysed for Urea and Creatinine.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Drugs/Chemicals used</th>
<th>Dosage (body weight.)</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dexamethasone</td>
<td>2mg/kg</td>
<td>Oral</td>
</tr>
<tr>
<td>2.</td>
<td>NaCl</td>
<td>4%</td>
<td>Oral</td>
</tr>
<tr>
<td>3.</td>
<td>Lisinopril</td>
<td>0.07mg per kg</td>
<td>Oral</td>
</tr>
<tr>
<td>4.</td>
<td>Amlodipine</td>
<td>0.27mg per kg</td>
<td>Oral</td>
</tr>
<tr>
<td>5.</td>
<td><em>Moringa oleifera</em></td>
<td>20mg and 50mg/kg</td>
<td>Oral.</td>
</tr>
</tbody>
</table>

**Table 1: Drugs/Chemicals**

**Group I:** Normal saline (negative control).

**Group II:** Induced dexamethasone-salt hypertension (positive control).

**Group III:** Induced dexamethasone-salt hypertension and treated with 0.07mg/kg of Lisinopril and 0.27mg/kg of Amlodipine.
Group IV: Induced dexamethasone-salt hypertension and treated with 20mg/kg of *Moringa oleifera* leaves extract.

Group V: Induced dexamethasone-salt hypertension and treated with 40mg/kg of *Moringa oleifera* leaves extract.

Group VI: Induced dexamethasone-salt hypertension and treated with 0.07mg/kg of Lisinopril, 0.27mg per kg of Amlodipine and 20mg/kg of *Moringa oleifera* leaves extract.

**Acute Toxicity Test**

Acute toxicity testing of *Moringa oleifera* was not carried out because it had already been determined to be above 1585mg/kg body weight by [24]. Dietary supplement capsules known as Nature’sfield® High strengthen Moringa 800mg were used.

**Sample Collection and Preparation**

During the experiment, about 5ml of blood samples were collected by Rectro-orbital plexus at weekly intervals; and at the end of the experiment euthanized with chloroform in a gas chamber and blood collected from the heart into sterile plain containers. The blood was allowed to clot, retracted and centrifuged at 4000g for 5minutes. The sera were separated and transferred into cryovials and stored (frozen) at -20°C until required for analysis.

**Biochemical Analysis**

The sera collected were analyzed with Cobas C111 Chemistry auto-analyzer at the Chemical Pathology Laboratory of Jos University Teaching Hospital (JUTH), Jos. This Cobas C111 works base on Beer-Lambert principle, which states that the amount of energy absorbed or transmitted by a solution is proportional to the solution’s molar absorptivity and the concentration of the solute. Sera obtained were used for the estimation of Urea and Creatinine.

**Statistical Analysis**

The data obtained was analysed using Statistical Package for Social Sciences (SPSS) version 25.0. The results were expressed as mean ± SEM. Group comparisons was done using one-way analysis of variance (ANOVA), p-value less than or equal to 0.05 (p≤0.05) was considered statistically significant.

**Results**

Table 2: Mean Concentrations and Standard Deviation of Serum Urea and Creatinine of Albino Rats Induced with Hypertension and Treated with Lisinopril, Amlodipine and *Moringa oleifera*

<table>
<thead>
<tr>
<th></th>
<th>Gp I ±SD</th>
<th>Gp II ±SD</th>
<th>Gp III ±SD</th>
<th>Gp IV ±SD</th>
<th>Gp V ±SD</th>
<th>Gp VI ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 2</td>
<td>8.08±0.69 (n = 5)</td>
<td>9.02±1.37 (n = 5)</td>
<td>6.80±0.10 (n = 3)</td>
<td>7.70±1.65 (n = 3)</td>
<td>7.77±2.63 (n = 3)</td>
<td>13.18±1.36 (n = 4)</td>
</tr>
<tr>
<td>Wk 3</td>
<td>6.84±0.96 (n = 5)</td>
<td>7.26±0.82 (n = 5)</td>
<td>7.67±0.32 (n = 3)</td>
<td>8.63±0.90 (n = 3)</td>
<td>8.63±2.27 (n = 3)</td>
<td>10.95±2.08 (n = 4)</td>
</tr>
<tr>
<td>Wk 4</td>
<td>7.60±0.85 (n = 5)</td>
<td>8.50±1.67 (n = 5)</td>
<td>7.23±0.68 (n = 3)</td>
<td>7.63±1.50 (n = 3)</td>
<td>6.63±1.32 (n = 3)</td>
<td>7.70±2.25 (n = 3)</td>
</tr>
<tr>
<td><strong>Cr (µmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wk 2</td>
<td>41.98±4.21 (n = 5)</td>
<td>32.88±1.99 (n = 5)</td>
<td>27.37±2.45 (n = 3)</td>
<td>29.13±2.39 (n = 3)</td>
<td>33.17±6.40 (n = 3)</td>
<td>31.78±3.50 (n = 5)</td>
</tr>
<tr>
<td>Wk 3</td>
<td>38.80±3.49 (n = 5)</td>
<td>30.58±4.09 (n = 5)</td>
<td>28.07±7.00 (n = 3)</td>
<td>27.67±3.00 (n = 3)</td>
<td>32.63±0.40 (n = 3)</td>
<td>30.35±5.19 (n = 5)</td>
</tr>
<tr>
<td>Wk 4</td>
<td>31.18±3.83 (n = 5)</td>
<td>22.34±7.57 (n = 5)</td>
<td>33.20±8.94 (n = 3)</td>
<td>29.90±5.81 (n = 3)</td>
<td>25.03±5.45 (n = 3)</td>
<td>25.03±5.20 (n = 5)</td>
</tr>
</tbody>
</table>

Key: Mean ± Standard Deviation = ±SD, Sample size = n, Creatinine = Cr. Week = Wk

Table 3: General Observations of Experimental Animals

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENT GIVEN</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water</td>
<td>Fur appearance Smooth, Bleeding Absent, Alert/activeness Active</td>
</tr>
<tr>
<td>II</td>
<td>Dexamethasone-salt</td>
<td>Slightly rough, Present, Less active</td>
</tr>
</tbody>
</table>
Table 3 summarizes the general observations of rats and various treatments. In group 2 (positive control), the fur appearance was slightly rough, bleeding from the nose, eyes and mouth in the second week of the experiment which was absent in the subsequent weeks.

Table 4: Percentage Mortality of Rats Induced Dexamethasone-Salt Hypertension and Treated with Lisinopril, Amlodipine and *Moringa oleifera*

<table>
<thead>
<tr>
<th>WEEKS</th>
<th>EXPERIMENTAL GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I n (%)</td>
</tr>
<tr>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Key:** n = number of rats, % = Percentage mortality,

Discussion

Table 2 above shows the mean serum urea concentrations of rats in Group III treated with Lisinopril and Amlodipine (6.80±0.10mmol/L, 7.67±0.32mmol/L, 7.23±0.68mmol/L) during the three weeks of the experiment; lower than those of Group I (8.08±0.69mmol/L, 6.84±0.96mmol/L, 7.60±0.85mmol/L). However, the concentrations of urea between Groups III and I was statistically insignificant with P-value 0.29. Mean creatinine
concentrations were also lower than that of negative control (Group I) with mean values 27.37±2.45µmol/L, 28.07±2.00µmol/L, 33.20±8.94µmol/L for Group III as compared to Group I with 41.98±4.21µmol/L, 38.80±3.49µmol/L, 31.18±3.83µmol/L respectively with a P-value 0.84. This is in agreement with the findings in a study by [25], who found that Amlodipine causes no significant changes in Creatinine and Urea, indicating the absence of nephrotoxicity (renal impairment); since high levels of Urea and Creatinine as markers are indicators of kidney dysfunction. This is also in agreement with the study of [26], who observed no significant difference in Creatinine levels of patients treated with Amlodipine and Lisinopril for four to eight weeks of their experiment. However, the findings of this work are in contrast with that of [27], who discovered a significant increase in serum Creatinine levels for the period of 21 days with a rise in Creatinine not statistically significant and a significant decrease in Urea, though statistically not significant in animals treated with Lisinopril.

Urea levels in Groups IV and V that were treated with Moringa oleifera at 20mg/kg and 40mg/kg bw respectively, were 7.70±1.65mmol/L, 8.63±0.90mmol/L, 7.63±1.50mmol/L for Group IV being higher than Group V which had 7.77±2.63mmol/L, 8.63±2.27µmol/L, 6.63±1.32µmol/L; with no statistically significant difference with a P-value of 0.29. There was also increase in the serum creatinine concentration but the concentration of serum creatinine was statistically insignificant with P-value of 0.84. This disagrees with the work of [28], who observed that there was a significant increase in Urea and Creatinine levels in rats that received Moringa oleifera at different doses. The Urea levels in Group I which is the positive control were 9.0±1.37 mmol/l, 7.26±0.82 mmol/l, 8.50±1.67 mmol/l were higher when compared to Group I which was the negative control which had 8.08±0.69mmol/l, 6.84±0.96 mmol/l, 7.60±0.85 mmol/l with a P-value of 0.109. There was no significant decrease in Creatinine levels of Group II which were 32.88±1.99µmol/l, 30.58±4.09µmol/l, 22.34±7.5µmol/l when compared to Group I which had 41.98±4.21µmol/l, 38.80±3.49µmol/l, 31.18±3.83µmol/l with a P-value of 0.617. This disagrees with the results of [29] who noticed significant increase in blood Urea and Creatinine levels in hypertensive albino rats.

The general behaviour and activity of animals during the experiment in the negative control Group (I) did not record any abnormal behaviour. They had normal fur appearance, and were generally active and alert. The rats in Group II which was the positive control Group showed significant changes when compared to other groups, with slightly rough fur appearance, bleeding from the eyes, nose and mouth and were less active. These observations are in line with the folktales by [30], who stated that one of the side effects of Dexamethasone is unusual tiredness, dizziness and hypertension. This also is in agreement with the findings of [31], who stated that plasma leptin may play a role in Dexamethasone-induced anorexia (eating disorder) which can lead to dizziness and weakness. In Groups III, IV, V and VI, there were significant changes when compared to Group I, with slightly rough fur appearance, bleeding from the eyes, nose and mouth in addition to being less active in week 2 of the experiment. Treatment with Lisinopril, Amlodipine and Moringa oleifera showed that animals returned to normal behaviour, which indicated resolution of hypertension.

Table 4 shows the death rates recorded in test Groups III, IV, V and VI with 1 death recorded in groups III and IV and 2 deaths were recorded in group V in week 1. This could be because of the intake of 0.4% sodium chloride for 5 days. This finding agrees with that of [32] who noticed that high level of sodium intake caused death in albino rats. In week 2 of the experiment, one mortality was recorded in Groups III, IV and VI each. In week 3 of the experiment, no death was recorded and in week 4 of the experiment, one mortality was recorded in Group VI. It is not clear the cause of death, but it may not be due to the drugs or Moringa oleifera. Studies of [24], agrees that aqueous leaf extract of Moringa oleifera is relatively safe when administered orally. Also, the mortality may not be due to Lisinopril and Amlodipine treatment [33], whose doses have been established [34].

In figure I, it was observed that the rats in Groups II, III, IV, V and VI showed decrease in body weight in week 1 of the experiment. This is in agreement with the studies by [31] and [35] who reported decrease in body weight of rats after administration of Dexamethasone but in contrast with the findings of [36], who reported increased body weight after administration of Dexamethasone-salt. In Group II, there was marked increase in body weight when the dexamethasone-salt inducing agent was withdrawn. This confirms the folktales by [37], who stated that reduction in sodium intake can reduce blood pressure and increase body weight. In Group II, from weeks 3-4, there was progressive decrease in body weight. This is in agreement with the folktales by [38], who stated that there is weight loss/decrease in high blood pressure. There was progressive increase in body weight from weeks 2-4 in Group III. This agrees with the report by [39], who observed weight gain in people taking Amlodipine; although it is rare and in contrast with the findings by [40], who observed that Lisinopril led to reduction in body weight. In Groups IV and V, there was increase in body weight in weeks 2 and 4 after treatment with Moringa oleifera had commenced. This could be due to the antihypertensive effects of Moringa.
Moringa oliefera on the Dexamethasone-salt induced hypertension. This is in agreement with the studies of [41], and [28], who observed increased body weight of animals treated with Moringa oliefera. However, the decrease in weight observed in Group IV in week 3 cannot be explained, though it seems to be similar to earlier findings by [42], who observed decrease in body weight of rats fed with aqueous extract of Moringa oliefera leaves. Group VI which received the conventional drugs and a low dose of Moringa oliefera showed progressive increase in body weight from weeks 2-4 which could be an indication of hypertension resolution, with subsequent return to normal life.

Conclusion
This study was designed to investigate the combined effects of antihypertensive drugs (Lisinopril and Amlodipine) and Moringa oliefera on the serum Urea and Creatinine levels in Albino Wistar rats. The findings of this study showed that Lisinopril, Amlodipine and Moringa oliefera (20mg/kg and 40mg/kg) showed no significant increase in levels of serum Urea and no significant decrease in levels of serum Creatinine. This indicates synergy in this class of antihypertensive drugs and this herb Moringa, at the said concentrations. Based on the results of this work, it can be concluded that Moringa oliefera leaves extract can be used complementarily along with Lisinopril and Amlodipine for the treatment and management of hypertension.

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Conflict of Interests
The authors declare no conflict of interest.

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