Evaluation of in Vivo Diuretic Activity of Methanolic Extracts of Clutia Abyssinica (Euphorbiaceae) Roots in Wistar Albino Rats

Abebaw Tegegne, Bharat Mishra and Mestayet Geta

1Ministry of Health, Jeddah, KSA
2King Abdullah Medical City (KAMC-HC), Makkah, KSA

Corresponding Author: Abebaw Tegegne
Dr-soso06@hotmail.com

ABSTRACT

Introduction: Most parts of the plant Clutia abyssinica is traditionally used in different diseases because of their medicinal properties. The roots are widely used traditionally, as diuretic purpose, liver problems, enlarged spleen and kidney problems. The present study was carried out to evaluate the diuretic effect of methanolic extracts of Clutia abyssinica roots in rats in comparison with standard drug furosemide. Methodology: Thirty rats of either sex were randomly allocated into five groups of six each. The rats were pretreated with physiological saline (0.9% NaCl, po) at a dose of 25 ml/kg, to impose uniform water and salt load. The control group received normal saline (20 ml/kg po), the standard group received furosemide (10mg/kg, po) and the test groups were administered different doses of the crude methanolic extract (100, 200 and 400mg/kg, po) respectively and urine was collected from individual rat for a period of 24 hrs. The urine volume and concentration of urine electrolytes were measured. Result: At medium (M200) and maximum (M400) doses of Clutia abyssinica had a significant urine output at the end of 6 hr (p<0.001) and 24hr (p<0.05) when compared with control group. Effect on electrolyte excretion showed in both 6hr and 24hr had an increased natriuresis (p<0.001 for both time of collection) and kaliuresis (p<0.05 for 6hr and p<0.001 for 24hr urine) at M400 dose, while the kaliuresis effect was smaller at M100 doses when compared with standard. The plant was also found to be safe at a maximum dose of 2000 mg/kg for methanolic extracts. Conclusions: These findings indicate that crude extract of Clutia abyssinica roots has good diuretic activity on rat model. However, future studies should focus on its exact mechanisms of action and isolating the phytochemical component(s) responsible for diuresis.

Keywords: Diuretics, Clutia abyssinica, natriuretic index, saluretic index

1. INTRODUCTION

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations\(^1\). The aim of most clinically use full diuretics to reduce extracellular fluid volume by decreasing total-body NaCl contents\(^2\). Diuretics not only alter the excretion of sodium (Na\(^+\)), but also may facilitate excretion of other cations (e.g., potassium (K\(^+\)), H\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\)), anions (e.g., Chloride (Cl\(^-\)), HCO\(_3\)\(^-\), and H\(_2\)PO\(_4\)\(^-\)), and uric acid [3]. Many indigenous plants have been claimed to have diuretic effect in ethno botanical study on traditional medicinal plants but lack scientific authentication. Clutia abyssinica roots...
are widely used traditionally, for diuretic purpose\(^4\) and kidney problems\(^5\). The present study was provided scientific evidence for the efficacy of Clutia abyssinica extract in diuretic activity of the plant.

2. METHODS

**Material collection**

Fresh roots of Clutia abyssinica were collected around Gondar town, North West Ethiopia during the month of January 2016. Taxonomic identification was done and a voucher specimen (Voucher No. 001) was deposited at the National Herbarium, Addis Ababa University. The roots were thoroughly washed with tap water to remove dirt, soil and any other foreign materials.

**Experimental animals**

Thirty wistar albino rats of either sex weighing 100-120 g, 8-12 weeks of age, obtained from animal breeding house of School of Pharmacy, University of Gondar. were used. Each rat were housed in plastic box cage under standard environmental conditions and was kept under 12 hr/12 hr light/dark cycle with free access to standard pellet feed obtained from Ethiopian Health and Nutrition Research Institute, Addis Ababa.

**Extraction**

Eight hundred grams of the dried powder of roots was macerated with about 1.2L of 80% methanol for 72 hr. The filtrate was separated from the marc by using tea sieve followed by filter paper (Whatman No.1) and then the marc was re-macerated twice using the same volume of solvent to exhaustively extract the plant material. The methanol was removed from extract by evaporation with heating on oven at 40^\circ C. The dried extract was weighed and calculated for percentage yield, which was 10% (w/w) and the dried extract was stored in a desiccator until the actual experiment\(^6\).

**Phytochemical screening**

The crude methanol extract was assessed qualitatively for secondary metabolites such as alkaloids, tannins, glycoside, steroid, terpenes, flavonoids, spooning and anthraquinones using standard methods\(^7\).

**Assessment of oral acute toxicity**

The methanolic extract of Clutia abyssinica roots was evaluated for its toxicity in female Swiss albino mice at the age of 8-12 weeks, by using the limit test dose of 2000 mg/kg body weight according to OECD guidelines No 425\(^8\). After administration of the extract, the mice were carefully observed continuously for behavioral, neurological, autonomic or physical changes for the first 4 hr and then for the next 24 hr. Thereafter, they were kept under close observation up to 14 days to monitor the presence of any signs of morbidity or mortality.

**Grouping and dosing of animals**

The rats of either sex were randomly assigned into five groups each comprising of six for diuretic test. Group I- Negative controls [Normal saline 20 ml/kg (control)]. Group II- Positive controls [Furosemide 10 mg/kg (standard)]. Group III- Treated with M100 (Clutia abyssinica 100mg/kg). Group IV-Treated with M200 (Clutia abyssinica 200mg/kg) and Group V- Treated with M400 (Clutia abyssinica 400mg/kg). The doses were determined based on the acute toxicity studies as per OECD guidelines\(^8,9,10\). After the safety of the extract was determined, 1/10th of the maximum dose was considered as a middle dose (200mg/kg), half and double of the middle doses were selected and considered as low and high dose, (100mg/kg and 400mg/kg) respectively. The test doses were prepared freshly on the day of the experiment.

**Assessment of diuretic activity**

Diuretic activity was carried out in accordance with slight modification of the earlier method \[10\]. Thirty, either sex rats were randomly allocated into five groups of six each and food was restricted for 18 hours prior to the experiment, but free access to water was allowed. The rats were pretreated with physiological saline (0.9% NaCl) at an oral dose of 25 ml/kg body weight, to impose a uniform water and salt load\(^10\). Each group was then treated as described in grouping section above orally. Immediately after administration, the rat was individually placed in metabolic cages provided with a wire mesh bottom and a funnel to retain faeces and to allow the urine to pass. The supplement of water was withdrawn during experiment time (24 hr).

**Urine volume**

The urine was collected and measured at 2, 4, 6, and 24 hr after dosing, then stored at −20^\circ C until electrolyte analyses\(^11,12\). The following parameters were calculated in order to compare the effects of the extracts with vehicle and standard on urine excretion. The urinary excretion was calculated as the ratio of total urinary output to total liquid administered. The ratio of urinary excretion in test group to urinary excretion in the control group was used as a measure of diuretic action of a given dose of extract. A parameter known as diuretic activity was also
calculated. To obtain diuretic activity, the diuretic action of the extract was compared to that of the standard drug in the test group\(^{(13)}\). Lipschitz’s value was determined as a ratio of urine volume of test group and urine volume of standard group. Diuretic index was the ratio of urine volume of test group and urine volume of control\(^{(12)}\).

**Urine Electrolytes concentration**

Urine samples by using a pH meter. Urine electrolytes (Na+, K+ and Cl-) concentrations (from six hour and twenty four hour collected urine) were estimated and expressed as mmol/L. Analytical estimation was performed according to the procedure provided along with electrolyte estimation standard reagents kit (Crest Biosystems, India). Urine Sodium and potassium were determined by using flame photometry, while chlorine was quantified by argentometrically with potentiometrical end point titration (Chloride-Titrator Aminco), by using silver nitrate and potassium chromate solution as titrant and indicator respectively. The sum of Na+ and Cl- was calculated as parameter for saluretic activity and the ratio of Na+/K+ was calculated for natriuretic activity\(^{(14,15)}\). The carbonic anhydrase inhibition was also evaluated as the ratios of urine electrolytes; Cl-/K++Na+.

**Statistical analysis**

Statistical work was done by using SPSS software version 20. Values were expressed as mean ± SEM. The statistical evaluation were carried out with one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Data were considered statistically significant if P value <0.05.

3. RESULT

**Oral acute toxicity**

Oral acute toxicity of methanolic extract of Clutia abyssinica roots was evaluated in female Swiss albino mice. The acute toxicity study indicated that the extract was safe at a dose of 2000 mg/kg body weight. The extract didn’t cause for behavioral, neurological, autonomic or physical changes within 24 hrs. After 14 days of observation of the experimental mice, didn’t show any visible signs of morbidity and mortality.

**Phytochemical screening**

Phytochemical screening of the methanol extracts of Clutia abyssinica roots had revealed the possible presence of different secondary metabolites.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Methanol extract of C. abyssinica roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
</tr>
</tbody>
</table>

**Diuretic Activity**

**Effect on latency to first urination**

The graph of Figure 1 as shows below the latency to first urination of rats treated with the methanolic extract of Clutia abyssinica, control and standard groups. The first urination latency of extracts was 19.5 min (p < 0.01), 21 min (p<0.01), and 23.5 min (p<0.01) at doses 100, 200 and 400 mg/kg.

**Effect on Urine Volume**

The methanolic extracts produced diuresis which appeared to be dose-dependent manner (Table 2). The rats treated with M400 induced significant increase in urine volume starting from second hour (2.38±0.20, p<0.01) and continued until the end of twenty four hour (4.03±0.23, p<0.05), as compared to control group. M400 and M200 showed high significance (p<0.001) urine volume at the end of six hour when compared with control group. Rats treated with M100 has increased diuresis starting from the second hour of urine collection, but that was not found to be a significant diuresis at all time when compared with control group. Standard displayed a significant effect compared to M100 at second hour (p<0.01) and continued until the end of 6 hour (p<0.01), but not observed significance at the end of 24hr.
Effect on urinary excretion and related parameters

The rats treated with M200 and M400 of the methanolic extract of the Clutia abyssinica roots were produced urinary excretion (3.22%), diuretic action (1.32) and diuretic activity (0.78). The percent urinary excretion and diuretic action of the M100 (2.58%, 1.06) and control group (2.44%, 1.00).

The diuretic index values of the extract groups M100, M200 and M400 were 1.47, 1.79 and 1.83, respectively, whereas the standard diuretic index values was 2.08.

Table: 2. Effect of methanolic extract of Clutia abyssinica roots on urine volume in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/ kg po.)</th>
<th>2hr</th>
<th>4hr</th>
<th>6hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20ml</td>
<td>0.50±0.21</td>
<td>0.96±0.30</td>
<td>1.10±0.29</td>
<td>2.20±0.61</td>
</tr>
<tr>
<td>Standard</td>
<td>10mg</td>
<td>2.98±0.51ₐᵇ²</td>
<td>3.43±0.57ₐᵇ²</td>
<td>3.87±0.43ₐᵇ²</td>
<td>4.57±0.48ₐᵇ²</td>
</tr>
<tr>
<td>M100</td>
<td>100mg</td>
<td>1.40±0.19</td>
<td>1.52±0.16</td>
<td>2.12±0.16</td>
<td>3.23±0.16</td>
</tr>
<tr>
<td>M200</td>
<td>200mg</td>
<td>2.25±0.15ₐ²</td>
<td>2.53±0.17ₐ¹</td>
<td>3.18±0.13ₐ³</td>
<td>3.93±0.21ₐ¹</td>
</tr>
<tr>
<td>M400</td>
<td>400mg</td>
<td>2.38±0.20ₐ²</td>
<td>2.95±0.21ₐᵇ²</td>
<td>3.48±0.23ₐᵇ¹</td>
<td>4.03±0.23ₐᵇ¹</td>
</tr>
</tbody>
</table>

The Lipchitz’s value for each extract was also calculated and the results showed when, compared with standard, the M100, M200 and M400 of Clutia abyssinica showed 71, 86 and 88 % diuretic activity, respectively (Table 3).
**Table 3:** Effect of methanolic extract of Clutia abyssinica roots on urinary excretion and Related Parameters in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/Kg po.)</th>
<th>Diuretic Index</th>
<th>Lipschitz’s Value</th>
<th>% Urinary Excretion</th>
<th>Diuretic action</th>
<th>Diuretic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20ml</td>
<td>1</td>
<td>_</td>
<td>2.44</td>
<td>1</td>
<td>_</td>
</tr>
<tr>
<td>Standard</td>
<td>10mg</td>
<td>2.08</td>
<td>1</td>
<td>4.15</td>
<td>1.70</td>
<td>1</td>
</tr>
<tr>
<td>M100</td>
<td>100mg</td>
<td>1.47</td>
<td>0.71</td>
<td>2.58</td>
<td>1.06</td>
<td>0.62</td>
</tr>
<tr>
<td>M200</td>
<td>200mg</td>
<td>1.79</td>
<td>0.86</td>
<td>3.22</td>
<td>1.32</td>
<td>0.78</td>
</tr>
<tr>
<td>M400</td>
<td>400mg</td>
<td>1.83</td>
<td>0.88</td>
<td>3.22</td>
<td>1.32</td>
<td>0.78</td>
</tr>
</tbody>
</table>

**Fig. 2:** Time course of diuresis in rats treated with different doses of methanolic extract of Clutia abyssinica roots

**Effect on Urinary electrolyte excretions**

**Effect on six hour urinary electrolyte**

Effect of extracts on urinary K+ excretions during the period of 6hr collection was described below in Table 4. The maximum doses of extracts M400 (75.97±3.93) and M200 (85.33±2.40) were showed a statistical significance (p<0.05) on urinary K+ excretions in comparisons among M100 and control group.

In all extract groups, the Cl- excretion was significantly different when compared with control group (p<0.01, p<0.001, p<0.001); M100, M200, M400 respectively. The Cl- excretions of the standard group there was greatly significant increased (169.80±5.88, p<0.001) when compared with control group (115.00±7.64).

Saluretic activity of M400 extract was observed (246.67±0.78, p<0.001) and the standard (260.8±4.91, p<0.001). Effect on Saluretic activity of M200 extracts was significant (245.33±5.10, p<0.01) when compared with control. Saluretic activity of M100 was increased significantly (230.86±7.85, p<0.01) as compared to control, but significantly decreased (p<0.05) when compared with standard, M200 and M400 groups (Table 4).

Natriuretic activities of standard drugs (1.12) slightly higher than the control and the M200 extract dose.
group (1.05, 1.07 respectively), but lower than the M100 extract (1.41). On the other hand, the CAI activity of rats treated with M100 (0.99) was higher than all groups and the minimum value was also observed in the standard group (0.83). The CAI activity of the maximum dose of extract was 0.92, whereas the moderate dose was 0.91 (Table 4).

**Table: 4. Effect of methanolic extract of Clutia abyssinica roots on 6hr urinary electrolytes excretion in rat**

<table>
<thead>
<tr>
<th>Group</th>
<th>Urinary electrolyte concentration (mmol/L) on 6hr urine</th>
<th>Activity of Different parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>Control</td>
<td>63.10±3.52</td>
<td>60.10±10.26</td>
</tr>
<tr>
<td>Standard</td>
<td>103±1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>91.67±2.19&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>M100</td>
<td>85.43±3.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.67±1.20</td>
</tr>
<tr>
<td>M200</td>
<td>91.60±2.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.33±2.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>M400</td>
<td>96.80±3.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.97±3.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

(n=6) a: against control, b: against M100 1: p<0.05, 2: p<0.01, 3: p<0.001

**Effect on twenty four hour urinary electrolytes**

The estimated concentration of Na⁺, K⁺ and Cl⁻ (Table 5) excreted in 24hr urine by rats following treatment with Clutia abyssinica extract at M400 were statistically significant (p<0.001) as compared to control group. Rats treated with the extract dose of M200 had shown a significant effect (p<0.01, p<0.001, p<0.01 respectively) against control group on Na⁺, K⁺ and Cl⁻ excretion. M100 also showed significant effect (p<0.05) in Na⁺ and Cl⁻ excretion when compared with control group. Standard and M400 displayed a significant effect compared to M100 (p<0.01) in K⁺ urinary excretion. Effect of methanolic extract of Clutia abyssinica roots on saluretic activity (Na⁺ + Cl⁻) in 24hr urinary electrolyte were enhanced significantly (p<0.05, p<0.01, p<0.001) at M100, M200 and M400 respectively as compared with control group. Standard was showed great significant (p<0.001) on saluretic activity as compared to control group (Table 5). Carbonic anhydrase inhibitory activities measurement was done for different treatment groups of extract of Clutia abyssinica roots. The highest CAI activity was observed in the M100 group (0.87), whereas the minimum value was showed in the medium dose of extract (0.81). The CAI activity with maximum dose of extract showed similar effect with that of the standard group (0.82) (Table 5).

On the other way natriuretic activities (Na⁺/K⁺) with M100 (1.43) was higher than control (1.14), standard (1.15), M200 (1.19) and M400 (1.24).

**Table: 5. Effect of methanolic extract of Clutia abyssinica roots on 24hr urinary electrolytes excretion in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Urinary electrolyte concentration (mmol/L) on 24hr</th>
<th>Saluretic Activity</th>
<th>Natriuretic Activity</th>
<th>CAI activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
<td>Cl⁻</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>103.27±5.63</td>
<td>90.28±3.2</td>
<td>163.33±4.30</td>
<td>266.60±6.32</td>
</tr>
<tr>
<td>Standard</td>
<td>168.28±2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.28±1.86&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>260.13±2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>428.41±4.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M100</td>
<td>135.16±3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.34±2.3</td>
<td>200.75±5.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>335.90±5.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M200</td>
<td>148.97±2.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.5±2.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>221.53±3.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>370.50±6.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M400</td>
<td>158.09±3.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.64±3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>233.90±4.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>391.99±3.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

(n=6) a: against control, b: against M100 1: p<0.05, 2: p<0.01, 3: p<0.001.

4. DISCUSSION

Diuretics are drugs which altering the physiological renal tubular reabsorption of water and electrolytes into the blood stream, which facilitate the flow of urine together with greater excretion of electrolytes in the urine\(^\text{(16)}\). Hence, in the present study, both urine electrolyte concentration and urine volume parameters were measured to evaluate the diuretic effect of extract in rats.

The present study showed that the extract produced an orally active diuresis effect. Urine volume can be affected by different factors, which are the GFR, RBF and the degree of tubular re-absorption\(^\text{(2,17,18)}\). It might be proposed that Clutia abyssinica roots extract exerted diuretic effect by promoting vasodilatation in the afferent arterioles of the renal vasculature and thus increasing GFR. It is also possible that like ADH secretion inhibitory effect mechanisms, which causes polyurea. Another possible mechanism involved might be stimulation of release of endogenous natriuretic peptides, which contribute antagonism of ADH action in the collecting ducts and antagonism of Na+ reabsorption in multiple nephron segments by inhibiting aldosterone effects\(^\text{(19)}\).

At the minimum dose of extracts was not found to have significance effect. Previous studies in different plants for diuretic activity indicated that at the minimum dose produced insignificance diuresis\(^\text{(14,15,11,12)}\). It is therefore possible to suggest that the ingredient (s) of the plant material responsible for the flow of urine could possibly lack of enough concentration at these lower doses.

In addition, if the diuretic index value is > 1.50, it shows a good diuretic activity, whereas the diuretic index values ranging from 1.00–1.50 and 0.72–0.99 demonstrate moderate and mild diuretic activity, respectively and diuretic index value < 0.72 indicates no diuretic activity\(^\text{(20)}\). In the present study, the diuretic index values of the two maximum doses (1.79, 1.83) were as closer to standard drug (2.08) and > 1.50. So that, possible to put forward that the ingredient (s) of the Clutia abyssinica roots responsible for good diuresis effect on urine volume.

Furthermore, latency of the first urination of the diuretic action of Clutia abyssinica root extract was sufficiently rapid than control and standard groups. It was observed that; the lowest dose of extract had a slight difference latency of first urination than other extract. Control group latency of first urination was increased by 3 folds when compared to standard and extract groups. According to Ntchpda et al. reportes the effect of the aqueous extract of leaves of Cassia occidentalis on latency of first urination of rats sufficiently rapid than control and also at maximum dose of 400mg/kg slight higher than of standard groups. The urination latency of control groups increases by 2 fold compared with in all groups\(^\text{(21)}\). It might be suggested that the gastrointestinal absorption characteristics of active ingredient(s) of Clutia abyssinica roots possibly a quick absorption from the intestine. Methanolic extract of Clutia abyssinica roots had also a reasonably long duration of action especially at its maximum dose produced significance urine volume from the second hour (p<0.01) to the twenty four hour (p< 0.001). This type of action is therapeutically advantageous to reduce frequency of administration. The difference in the time of duration of the diuretic action of the different doses of the extracts may be related to the concentration and gastrointestinal absorption characteristics of the ingredient (s) of the Clutia abyssinica, which is/are responsible for the evidenced onset and duration of action of diuretic activity.

Loop diuretics like furosemide are very important in a number of conditions like hypertension, HF, liver cirrhosis. Furosemide, which act by inhibiting Na+/K+/Cl- co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na+, K+ and Cl- from the body\(^\text{(22,16)}\). In the present study, the extract was able to increase the volume of urine with statistical significance along with a considerable Na+, K+ and Cl- load which was comparable to that of standard in the 24hr. Hence, Clutia abyssinica roots extract at maximum dose seems to be share loop diuretics like furosemide mechanisms, which enhance Na+, K+ and Cl- excretion in urine.

The minimum dose of (M100) extract was showed that diuresis effect on excretion of urinary Na+ and Cl- with significant increases as compared to control group in 6hr (p<0.01) and 24hr (p<0.05) urinary electrolyte excretion. But kaluresis activity of M100 significantly (p<0.01, p<0.05, in 6hr and 24hr respectively) decreased as compared to standard and the maximum extract dose. The increase in the concentration of excreted Na+ and decrease excreted K+, which is a very essential quality of good diuretic with lesser hyperkalemia side effect\(^\text{(23)}\). The chronic use of some diuretics may require the oral administration of potassium supplements or potassium saving diuretics that reduce urinary K+ excretion\(^\text{(24)}\).

Based on these findings, it is hypothesized that the diuretic action of extract might be the consequence of...
inhibition of epithelial sodium channels or aldosterone action at low dose. Natriuretic activity (Na+/K+) greater than 2.0 indicate a favorable natriuretic effect, and the ratios greater than 10.0 indicate a K+-sparing effect\(^\text{(10)}\). The present study was indicated that the M100 treated group increase sodium excretion more than potassium; as compared to other extract treated groups and standard. As a result natriuretic activity of M100 (1.43) extract in 24hr ,which was relatively more than furosemide(1.13), it indicated that the weak kaliuresis or K+ saving property of the extract, exhibited advantageous effect with respect to hypokalemia, one of the potential adverse effects of furosemide by acting on TAL of Henle. With reference to lower K+ excretion at a low dose of extract different from furosemide which significantly produced kaliuresis, so the mechanism was unlikely to be the loop diuretics type and also not likely to be as thiazide like type mechanism, due to these diuretic relatively increase the urinary K+ level with high Na+ load reaching the distal tube\(^\text{(25)}\).

In view of the saluretic activity of the plant extracts at maximum dose produced a comparable effect with that of standard in 6 hr and 24hr urinary electrolyte excretion. This indicated that the present studies of Clutia abyssinica extracts had a strong Na+ and Cl- excretion effect and long duration of action as comparable to standard. Furosemide has a high saluretic activity because it blocks the main sites of NaCl reabsorption at TAL\(^\text{(22,26)}\). As a result, it is suggest that the Clutia abyssinica roots extract at maximum doses might have a furosemide-like mode of action. Accordingly it is possible that the diuretic activity of extract might be elicited by inhibiting tubular reabsorption of water and ions, as such action has been hypothesized for the crude extract of the plant\(^\text{(38)}\).

The extent of CAI activity was calculated by using the formula Cl−/ Na++K+. CAI can be excluded at ratios between 0.8 and 1.0, but when decreasing the ratios; can be assumed that of slight to strong inhibition\(^\text{(10)}\). In the present study CA inhibitory activity at all doses of extracts did not observed (values above 0.8) in both 6hr and 24hr urinary electrolytes excretion. The extract active principle(s) responsible for the diuretic effects is/are so far unknown, but the preliminary secondary metabolite analysis of methanolic extract of Clutia abyssinica roots showed the possible presence of alkaloids, saponins, flavonoids, anthraquinones, phenol, glycoside and tannins. Previous studies in different plants have also demonstrated several compounds which could be responsible for the diuretic effect, such as flavonoids, phenol, saponins and alkaloid\(^\text{(11,12,13,27,28)}\). Such findings support that the presence of these secondary metabolites might be responsible for the diuretic activity of Clutia abyssinica roots, which they may act individually or synergistically. Saponins isolated from various plants inhibit the furosemide sensitive Na+-ATPase that has been demonstrated in proximal convoluted tubule of the kidney, which is responsible for trans-cellular sodium reabsorption\(^\text{(29,30,31)}\). Saluretic activity of extracts was observed that highly significance (p<0.001) in both 6hr and after 6hr urine electrolyte concentration as compared to control. Therefore, the presence of saponins in Clutia abyssinica roots might be responsible for the more pronounced saluretic activity of extract.

5. CONCLUSION

The methanolic extract of Clutia abyssinica showed diuresis, natriuresis and kaliuresis effects comparable to the standard drugs. From the result of this study, it can be conclude that crude methanolic extract of Clutia abyssinica roots possesses a diuretic activity in rat model.

REFERENCES
