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Diuretic Potential of The 50% Ethanol-Methanol Extract of Trema Orientalis Leaves in Albino Rats

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ABSTRACT
The present study was undertaken to evaluate the diuretic potential of the 50% ethanol-methanol extract of Trema orientalis leaves in Albino rats. Diuretic potential of Trema orientalis was evaluated in albino rats (Rattus norvegicus) at a dose of 100mg/kg body weight for 14 days and the diuretic potential were examined at day 1, 3, 5, 10 and day 14. The effect of administration of 100mg/kg body weight Trema orientalis on serum and kidney electrolytes (Sodium, Potassium and Chloride ion) were investigated in albino rats. 40 male albino rats with average weight 100 ± 5.0g were divided into 3 groups. Group 1 contained 15 rats, treated with Furosemide and group 2 contained 15 rats, treated with extract of Trema orientalis and group 3 contained 10 rats and served as control. Both groups were fed with rat feeds and water for 14 consecutive days while the test groups (1 and 2) were daily administered with 1.0 ml solution (25mg/kg and 100mg/kg body weight) of Furosemide and leaves extract of Trema orientalis respectively. Rats in group 3 (control) were administered with 1.0ml distilled water daily. The results obtained from administrations of Furosemide and leave extract of Trema orientalis on the electrolytes (Sodium, Potassium and Chloride ion) shows a significant change (decreased) in serum and kidney electrolyte concentration(mEq/L) compared to the control. Findings from this resultshowed significant change in the pattern of the electrolytes concentration (mEq/L) which is an indication that leaves extract of Trema orientalis has diuretic potential on the animal model. Administration of Trema orientalis showed diuretic potential in the rats which is comparable to that of the diuretic drug (Furosemide) used. Trema orientalis is thus a potential diuretic agent.

Keywords: Diuretic, Ethanol-Methanol, Extract, Trema orientalis, Furosemide

I. INTRODUCTION

Trema orientalis is a species of flowering tree in the hemp family, Cannabaceae. It is known by common names, including Charcoal-tree, Indian Charcoal tree (GRIN, 2007), Pigeon wood (Malan and Notten, 2005) and in Hawaii, where it has become naturalized gunpowder tree or Nalita (Jahan and Sung, 2005).

The tree has various uses as a herbal medicine in a wide range of cultures (Malan and Notten, 2005). The leaves and the bark are used to treat coughs, sore throats, asthma, bronchitis, gonorrhea, yellow fever, toothache and as an antidote to general poisoning (Orwa et al., 2009; Eckman and Hines, 1993). A bark infusion is reportedly drunk to control dysentery and a leaf decoction is used to deworm dogs (Orwa et al., 2009). In recent pharmacological studies, an aqueous extract from bark has been shown to reduce blood sugar levels in an experimental animal model of diabetes mellitus, and may be useful for treating this disease (Dimo et al., 2006). Extracts from leaves of related species (Trema guineese and Trema micrantha) showed anti-inflammatory, anti-arthritic and analgesic activity in rodents (Barbera et al., 1992) suggesting that T. orientalis could produce similar results.

Antioxidant and antimicrobial activities of Trema orientalis was studied by Sarder (2008), where the antioxidant potentials of methanolic extract was determined on the basis of its scavenging activity, this was established to be 110.25g/ml, which indicated the strong antioxidant activities of the plant. In the case of the antimicrobial activity test, the extract was investigated for its effectiveness against both Gram-positive and Gram-negative bacteria in agar diffusion method. It was evident that extract was active against the bacteria at low concentration.

Also, iron chelating and antiradical activity has also been established in a methanol soluble fraction extracted from Trema orientalis (Salprima et al., 2011), the extract was reported to exhibit an antiradical activity 69.73%

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(scavengeractivity of the stable1,1-diphenil-2-picry hydrazyl (DPPH) free radical) that was almost similar to that of 1mm ascorbic acid. However, the aim of this research is to evaluate the diuretic potential of 50% ethanol-methanol extract of Trema orientalis leaves in albino rats.

II. MATERIALS AND METHODS

MATERIALS

Collection and identification of plant materials

The plant samples were collected from Kogi State University Staff quarters environs, Anyigba, Local Government Area of Kogi State Nigeria. The plant was authenticated by Dr. D.O. Aina of Biological Science Department, Kogi State University, Anyigba. They were air dried for one week and then pulverized into fine powder using mortar and pestle.

Animal

Forty (40) rats (Rattus norvegicus), of average weight of 100 ± 5.0g were obtained from laboratory animal sellerin Markurdi, Benue State, Nigeria. The animals were acclimatized to the experimental house at 27 ± 2 ºC for a period of 14 days before the commencement of the experiment.

Chemicals and Reagents

All chemicals and reagents were of analytical grade. Distilled water, ethanol and methanol were product of BDH Pool, England.Normal saline, 0.25M sucrose solution, Sodium ion test kit, Potassium ion test kit and Chloride ion test kitwere product of TECO Diagnostics, USA.

Drug

Furosemide (40mg tablet B.P.) used is a product of SANOFI ADVENTIS U.S.

METHODS

Preparation of plants extract

The method of cold extraction was used. A portion (140g) of the powdered leaf sample was weighed and soaked in a jar containing 1000ml of 50% Methanol-Ethanol mixture for 24 hours. This was then filtered under vacuum pressure pump, which was later concentrated by removing the solvent completely using water bath. The concentrate yielded weighed 15.14g and was a greenish residue, which was kept in an air tightcontainer and kept in a refrigerator before use. Furthermore, the percentage yield was calculated to be 10.81%.

AnimalGrouping

The forty (40) albino rats (Rattus norvegicus) were divided into three groups:groups 1, 2 and 3. Group 1 and 2 contained 15 rats each, while group 3 contains 10 rats. Rats in group 1 were administered with 50% methanol-ethanol extract of Trema orientalis, rats in group 2 were administered with Furosemide (diuretic drug), while Rats in group 3 serves as control and they were administered with 1.0 ml of distilled water daily. All the rats were fed with standard rats feed and water ad libitum. The rats in each group were housed in ventilatedplastic experimental cages all through the period of the experiment.

Administration of drugs and plant extract

A stock solution of 10 mg/ml of 100 mg/kg body weight Trema orientalis leaves extract was administered orally for 14 days. Each ratin group 1 received 1.0 ml of the prepared plant extract daily, while a stock solution of 2.5mg/ml of 25mg/kg body weight Furosemide (diuretic drug) was also administered orally for 14 days in such a way that each animals in group 2, received 1.0 ml daily for 14 days. Day 1 represents rats that were sacrificed after first administration of plant extract and drug. Day 3 represents rats that were sacrificed after third day, which is 24 hours after third administration of both plant extract and drug. Day 5 represents rats that were sacrificed after fifth day of administration, while day 10 and 14 represent rats that were sacrificed 24 hours after tenth and fourteenth day of plant extract and drug respectively. The administration lasted for 14 days before they were sacrificed in their appropriated days. The rats in control group were only given ordinary water all through the period of the experiment.

Animal sacrifice and blood sample collection

Three rats in group 1 and 2 were sacrificed on day 1, 3, 5, 10 and day 14, after administration of appropriate doses. The rats were sacrificed by cervical dislocation and blood was collected from the jugular vein by cutting with sterile razor blade. The blood was immediately transferred into plain sample bottle and the blood was centrifuged at 4000rpm for 15 minutes. The supernatant obtained was kept in the refrigerator at -8ºC until required.

DETERMINATION OF ELECTROLYTE CONCENTRATION IN SERUM AND KIDNEY

Determination of Sodium ion Concentration

Sodium ion concentration was determined based on modification of the method described by Maruna(1958) and Trinder(1951), in which sodium is precipitated as the triple salt; sodium uranyl

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acetate, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide.

Determination of Potassiumion Concentration

The concentration of potassium ionwas determined based on the method of Maruna (1958) and Trinder (1951),in which sodium tetraphenyl boron is used in a specifically prepared mixture to produce a colloidal suspension.

Determination of Chlorideion Concentration

Chloride ion concentration was determined based on the modification method describe by Skeggs et al. (1967)in which chloride ion forms a soluble, non-ionized compound with mercuric ions and displace thiocyanate ions from non-ionized mercuric thiocyanate.

III. STATISTICAL ANALYSIS

Statistical analysis was carried out using student T-test at and the confidence level was set at 95%.

IV. RESULTS

Sodium ion (Na^+)

Table 1: Table showing Sodium ion (Na^+) concentration (mEq/L) in serum and kidney of rats administered with 100 mg/kg body weight of Trema orientalis and 25 mg/kg body weight of Furosemide (diuretic drug).

Tissue ⟶	Serum (Na^+) mEq/L		Kidney (Na^+) mEq/L	
	Furosemide	T. orientalis	Furosemide	T. orientalis
0	179.46	179.46	179.30	179.30
1	153.96	151.58	154.75	135.03
3	152.85	166.95	172.81	143.03
5	155.07	160.77	150.82	144.30
10	163.31	169.64	168.55	153.33
14	195.79	189.60	173.44	162.35

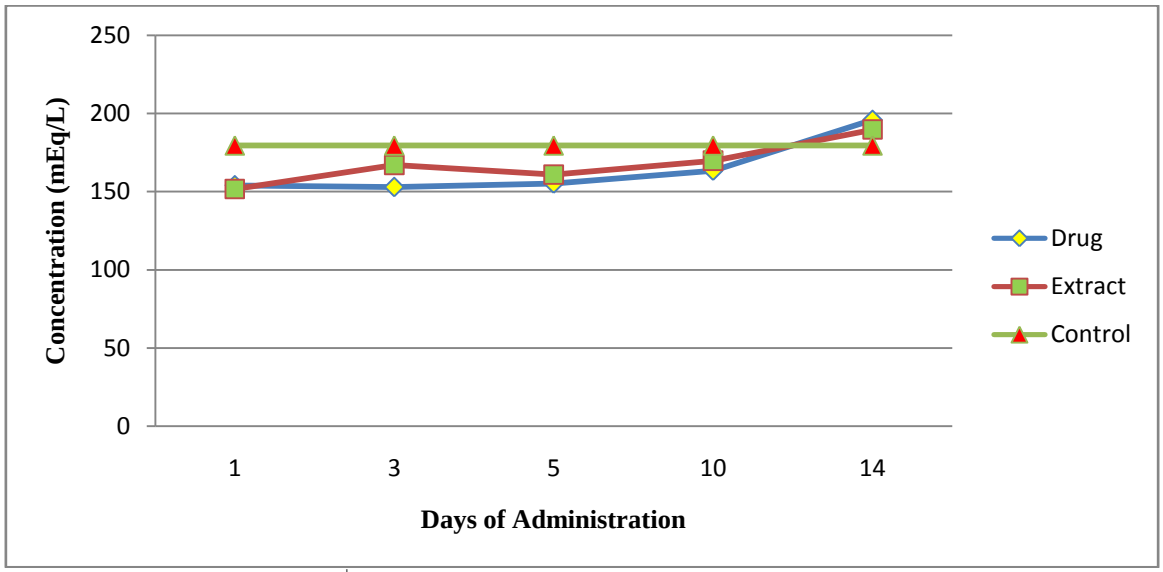

Figure1: Serum Sodium ion (Na^+) concentration (mEq/L) following administration of 100 mg/kg body weight Trema orientalis and 25 mg/kg body weight furosemide (diuretic drug)

Serum Sodium ion concentration of rats in drug group

Administration of Furosemide (25mg/kg body weight) to rats (Fig. 1) caused a marked decrease in serum sodium ion concentration on day 1 to day 3 compared with the control. There were 14% and 15% reduction respectively. Thereafter upon further administration the sodium ion concentration then showed gradual increase and even above the control value at day 14 of the experiment.

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Serum Sodium ion concentration of rats in extract group

Upon administration of Trema Orientalis (100 mg/kg body weight) to rats, there was a similar trend as observed on administration of furosemide following the initial administration (Fig. 1), when compared with the control. Thereafter, further administration let to a gradual increase in the serum sodium ion concentration. Though values were lower than control, this observed trends was maintained till day 10th dose, while after 14th dose, the serum sodium ion concentration increased above the control.

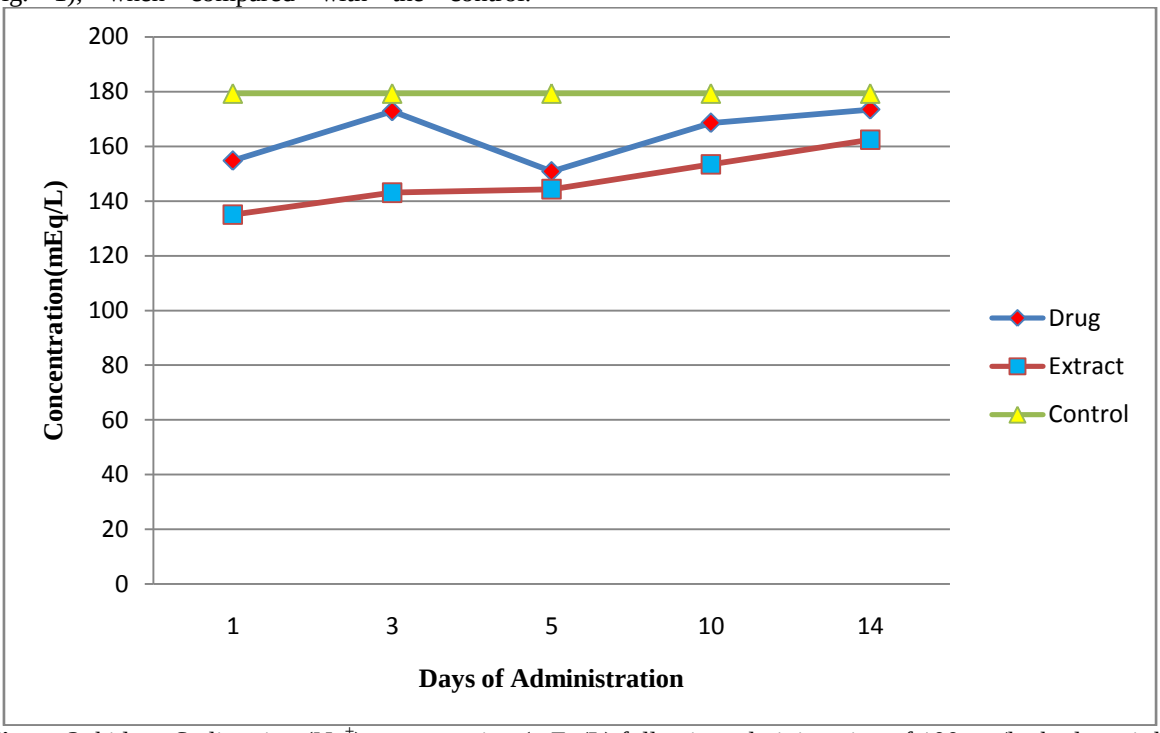

Figure 2: kidney Sodium ion (Na^+) concentration (mEq/L) following administration of 100 mg/kg body weight Trema orientalis and 25 mg/kg body weight furosemide (diuretic drug)

Kidney Sodium ion concentration of rats in drug group

Administration of Furosemide (25 mg/kg body weight) to rats (Fig.2) shows significant decrease in sodium ion concentration of kidney compared with that of the control on day 1 and the value increased towards that of the control on day 3. Though values on day 5 and 10 were lower than control, they show steady increase until the last day of the experiment, where the values were close to the control value. The kidney sodium ion concentration was observed to be lower when compared with that of the control at the experiment. At the last doses, the value for drug was higher (6.4%) than that for the extract group.

Kidney Sodium ion concentration of rats in extract group

The administration of Trema orientalis (100 mg/kg body weight) to rats (Fig. 2) shows a significant decrease in the kidney sodium ion concentration compared with that of the control throughout the period of experiment. The values increase progressively till the end of the experiment on day 14.

Potassium ion (K^+)

Table 2: Table showing Potassium ion (K^+) concentration (mEq/L) in serum and kidney of rats administered with 100 mg/kg body weight of Trema orientalis and 25mg/kg body weight of Furosemide (diuretic drug)

Tissue →	Serum (Na^+) mEq/L		Kidney (Na^+) mEq/L	
Days ↓	Furosemide	T. orientalis	Furosemide	T. orientalis
0	7.67	7.67	9.60	9.60

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1	7.31	4.95	1.65	2.09
3	6.32	5.61	3.05	2.05
5	3.41	4.72	2.84	2.65
10	3.81	4.48	5.38	3.77
14	4.53	5.34	8.60	8.06

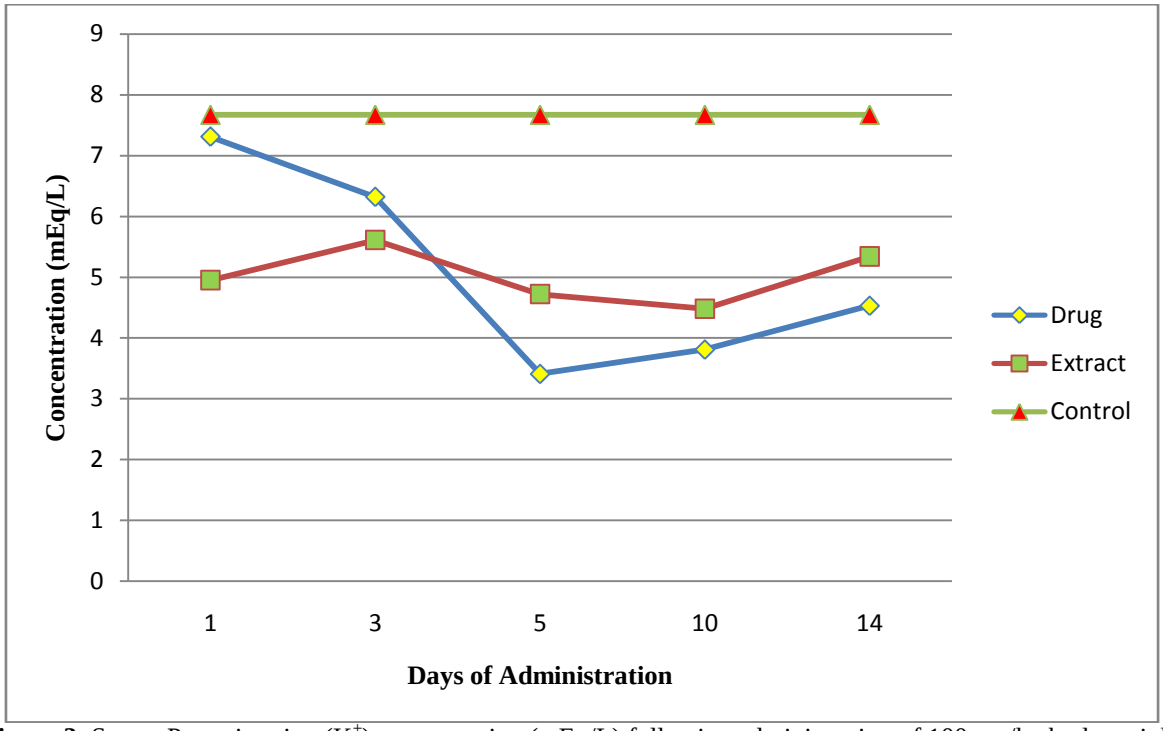

Figure 3: Serum Potassium ion (K^+) concentration (mEq/L) following administration of 100 mg/kg body weight Trema orientalis and 25 mg/kg body weight furosemide (diuretic drug).

Serum potassium ion concentration of rats in drug group

Upon the administration of 25mg/kg body weight Furosemide to rats (Fig. 3), there was a gradual decrease in serum potassium ion concentration from day 1 which persisted until day 10. Though there was an increase in the value on day 14, the value was not statistically different from value for day 10.

Serum potassium ion concentration of rats in extract group

Following the administration of 100 mg/kg body weight Trema orientalis to rats (Fig.3), there was a decrease in serum potassium ion concentration on day 1 when compared with both control and day 1 of drug group. The observed trend in the serum potassium ion concentration for the other days of the experiment i.e. day 3, 5, 10 and day 14 was similar to that observed with the drug group. Potassium ion concentration was observed to be lower than that of the control all through the experiment period.

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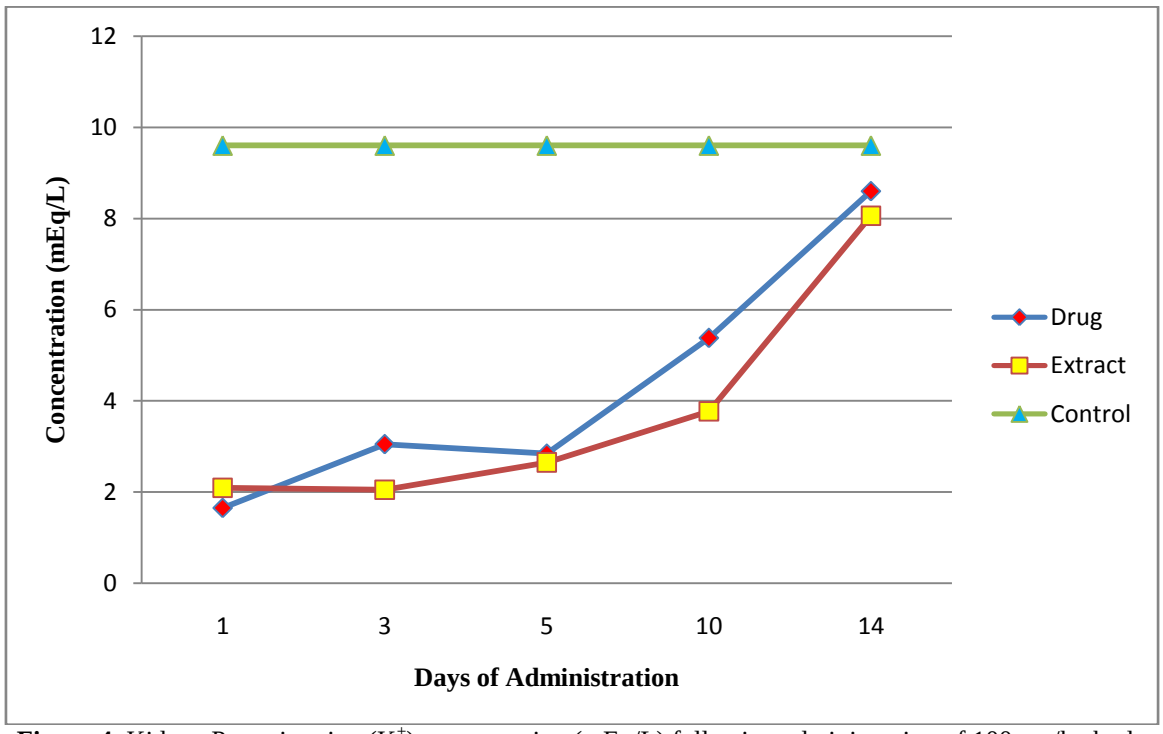

Figure 4: Kidney Potassium ion (K^+) concentration (mEq/L) following administration of 100 mg/kg body weight Trema orientalis and 25 mg/kg body weight furosemide (diuretic drug).

Kidney Potassium ion concentration of rats in drug group

Upon the administration of 25 mg/kg body weight Furosemide to rats (Fig. 4), there was a sharp decrease in the kidney potassium ion concentration on day 1 compared to the concentration of that of the control group and rose gradually as the experiment proceeds to the 14th day.

Kidney Potassium ion concentration of rats in extract group

The administration of 100 mg/kg body weight Trema orientalis to rats (Fig. 4) shows a decrease in kidney potassium ion concentration compared with the kidney potassium ion concentration of control throughout the experiment. It was observed that the potassium ion concentration decreased significantly first and thereafter start to increase gradually as the experiment proceeds.

Chloride ion (Cl^-)

Table 3: Table showing Chloride ion (Cl^-) concentration (mEq/L) in serum and kidney of rats administered with 100 mg/kg body weight of Trema orientalis and 25mg/kg body weight of Furosemide (diuretic drug) **.**

Tissue →	Serum (Na^+) mEq/L		Kidney (Na^+) mEq/L	
Days ↓	Furosemide	T. orientalis	Furosemide	T. orientalis
0	101.50	101.50	105.20	105.20
1	73.75	68.13	77.50	51.88
3	49.30	68.75	98.13	48.80
5	97.25	51.88	93.13	52.60
10	95.25	58.13	98.20	87.50
14	110.88	70.09	101.88	90.50

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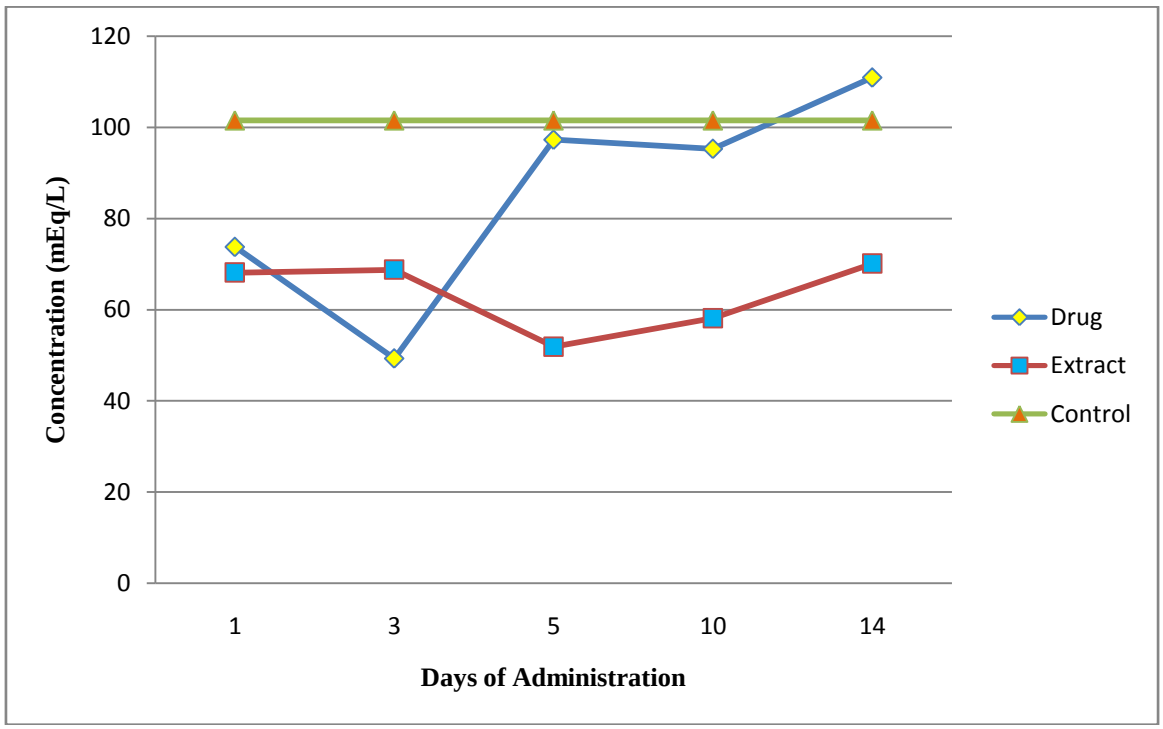

Figure 5: Serum Chloride ion (Cl^-) concentration (mEq/L) following administration of 100 mg/kg body weight Trema orientalis and 25 mg/kg body weight Furosemide (diuretic drug).

Serum Chloride ion concentration of rats in drug group

The administration of 25 mg/kg body weight Furosemide to rats (Fig. 5) shows a very significant decrease at day 1 compared with the control. A further decrease was observed at day 3. Day 5 and day 10 was observed to have chloride ion concentration above day 1 and at day 14, the chloride concentration increased above that of the control.

Serum Chloride ion concentration of rats in extract group

The administration of 100mg/kg body weight Trema orientalis to rat (Fig. 5) shows a decrease at day 1, 3, 5, and day 10 of the experiment when compared with the control. However, the serum chloride ion concentration increased slightly than the other previous days.

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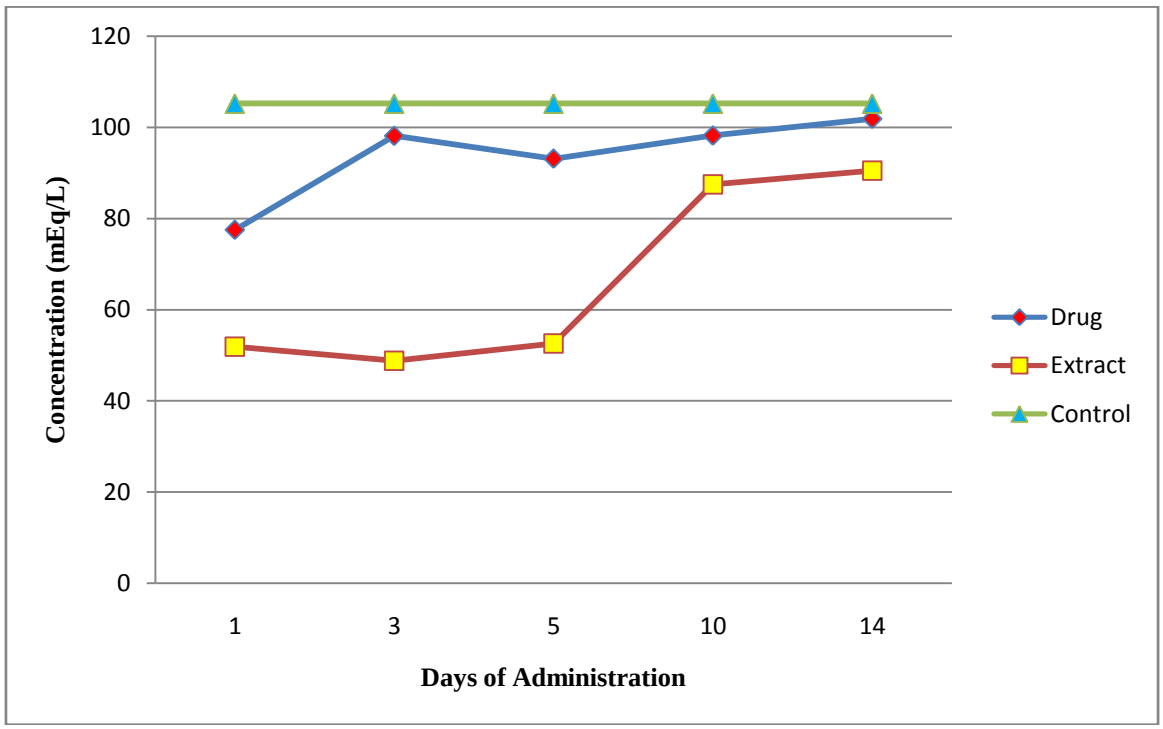

Figure 6: Kidney Chloride ion (Cl^-) concentration (mEq/L) following administration of 100 mg/kg body weight Trema orientalis and 25 mg/kg body weight Furosemide (diuretic drug).

Kidney Chloride ion concentration of rats in drug group

The administration of 25 mg/kg body weight Furosemide to rats (Fig. 6) showed a decreased chloride ion concentration of the kidney when compared with the control. It was also observed that, a trend of gradual increase of chloride ion concentration occur as the experiment proceeds i.e. the drug shows a dose-response.

Kidney Chloride ion concentration of rats in extract group

Administration of 100 mg/kg body weight Trema orientalis to rats (Fig.6) showed a significant decrease in the kidney chloride ion concentration on day 1 and day 3 when compared to control. Further administration resulted in gradual increase in concentration of Cl^- till the end of the experiment.

V. DISCUSSION

African medicinal plants have been widely accepted to have therapeutic potentials and thus serve as a major source of pharmaceuticals, food additives, fragrances and pesticides (Patwardhan et al., 2004). Consequently, a number of known natural products have been used in various traditional medical systems to obtain relief from symptoms of pain (Hossain et al., 2013).

In this work, the diuretic potential of 50% ethanol-methanol leaf extract was investigated by employing Furosemide, a known loop diuretic, as a comparative standard. The result of this work showed a close comparison in both serum and kidney sodium ion concentration between Furosemide and Trema orientalis leaf extract (Fig. 1 and 2). The line graphs for the two parameters are closely compared (Fig. 1 and 2). This may indicate similar level of inhibition of reabsorption of sodium ion by T. orientalis leaf extract as in Furosemide.

Similarly, the non-significant difference in the $[K^+]$ in the kidney after 14 days of administration of both the drug and the extract, 8.60 and 8.06 mEq/L respectively compared with the control tend to establish the diuretic compatibility of T. orientalis leaf extract (Fig. 4).

The observed serum value of $[K^+]$ for the leaf extract with the controls (5.34mEq/L and 7.67mEq/L) respectively may be due to the capacity of the extract to inhibit the reabsorption of potassium (Fig. 3) As a diuretic agent, following administration, delivery of sodium ion and filtrate to the cortical collecting duct (the major site of potassium secretion), would increase, leading to increased urinary potassium excretion (Fig. 4).

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The correlation between the chloride ion concentration values for the serum and kidney and its relationship to the concentration of both sodium and potassium ions may be indication of active reabsorption of NaCl and KCl via the Na^+-K^+-$2Cl^-$ symport.

The 50% ethanol-methanol leaf extract of Trema orientalis have close potentiality with Furosemide as diuretics. Nevertheless, furosemide was more potent as was revealed by the present study.

VI. CONCLUSION

In conclusion, the daily administration of 100mg/kg body weight 50% ethanol-methanol extract of the leaves of Trema orientalis to rats increased the excretion of Na^+, K^+andCl^- and causes a decrease in urine osmolarity. Data obtained from this result suggest that 50% ethanol-methanol leaves extract of T. orientalishas the potential of exerting diuretic activity and this justifies the traditional use of this plant in the treatment of Edema and Hypertention.

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