






Diuretic activity of ethanol extract of *Mirabilis jalapa* (Linn.) leaf in normal male Wistar rats



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Background: Ethanol extract of *Mirabilis jalapa* leaf (EEMJL) has been used in the folk medicine of Nigeria as diuretics without any scientific evidence.

Aim: Ethanol extract of *Mirabilis jalapa* leaf at 200, 400 and 600 mg/kg body weight was investigated for diuretic activity in male Wistar rats.

Setting: Fresh leaf of *M. jalapa* was collected from a farmland at the Alanamu area in Ilorin, Kwara State, Nigeria, authenticated and processed for the study.

Methods: Thirty male rats (231.50 g ± 13.51 g) were assigned into five groups (A–E) of six rats each. Rats in group A (control) received 1.0 mL of physiological saline (the vehicle). Animals in groups B (positive control), C, D and E received 1.0 mL equivalent to 100 mg/kg body weight of furosemide, 200, 400 and 600 mg/kg body weight of EEMJL, respectively. All administrations were done by oral gavage. The animals were monitored for indicators of diuresis for 5 h using standard methods.

Results: Ethanol extract of *Mirabilis jalapa* leaf dose-dependently increased ($p < 0.05$) urine volume, urine concentrations of Na⁺, K⁺ and Cl⁻ and decreased ($p < 0.05$) the body weight of the animals. Ethanol extract of *Mirabilis jalapa* leaf increased the urine pH, saluretic activity, saluretic index, Na⁺ index, K⁺ index, Cl⁻ index, diuretic action (diuretic index), kaliuretic index, Lipschitz value and percentage saline load excreted, whereas the latency of urination, natriuretic index, carbonic anhydrase inhibitory activity and carbonic anhydrase inhibition index were decreased. The EEMJL treatment-related changes in these parameters were essentially similar to those of the furosemide-treated animals.

Conclusion: This study has thus validated diuretic activity of *M. jalapa* leaf with the 600 mg/kg body weight of EEMJL being the most effective.

Keywords: Diuretic activity; Furosemide; *Mirabilis jalapa*; Nyctaginaceae; Safety.

Introduction

Diuretics augment the renal excretion of water and ions (sodium and either chloride or bicarbonate) by eliminating the excess fluid through enhanced excretion of urine (Barrar 2003). Drug-induced diuresis is beneficial in many life-threatening disease conditions such as congestive heart failure, hypertension, nephritic syndrome, cirrhosis, renal failure and pregnancy toxemia (Agunnu et al. 2005). Unfortunately, however, most of the orthodox diuretic agents, such as thiazides, loop and potassium-sparing, have many adverse effects that include, but not limited to, electrolyte imbalance, metabolic alterations, impotence, fatigue and weakness (Hullatti, Sharada & Kuppasth 2011), hence the need to search for newer and safer therapeutic diuretics in plants. Herbal drugs have gained importance and popularity in recent years because of their efficacy, cost-effectiveness and safety (Chauhan & Johnson 2009; Yakubu et al. 2018). Medicinal plants therefore serve as alternative sources for the development of new diuretics because of the presence of some chemical compounds that confer pharmacological activities on them.

Mirabilis jalapa Linn. (family: Nyctaginaceae), commonly known as Marvel of Peru or four O'clock plant (English) and in the Yoruba Language of South-western Nigeria as *Itanna paosho*, *Tanaposo* or *Tanapowo*, is a tall, perennial herbaceous climbing plant. The flowers can be white, red, pink, purple or multi-coloured, while the leaves are oppositely arranged. *Mirabilis jalapa* has a sweet fragrance and is a favourite ornamental garden plant that survives under diverse climatic conditions. The leaf is reputed for use in folk medicine as a diuretic, purgative, anti-diarrhoea, antimalaria, antipyretic, antidiabetic and suppuration for boils and abscesses (Gill 1992). Furthermore, the leaf have been claimed to be used for cleaning wounds, bruises and cuts and

for treating and/or managing inflammations, dropsy, constipation, dysentery, uterine discharges, muscular pain, abdominal colics, oedema, conjunctivitis and genitourinary disorders (Alonso & Ochoa 1999; Dimayuga, Virgen & Ochoa 1998; Holdsworth 1992; Lee, Xiao & Pei 2008; Weckerle et al. 2009). The root has also been acclaimed to be used as an aphrodisiac in folk medicine (Kirtikar & Basu 2001).

Several studies have been reported on the plant in the open scientific literature. *Mirabilis jalapa* has numerous phytoconstituents such as alkaloids, glycosides, carbohydrates, phytosterols, flavonoids, phenols, steroids, triterpenes, tannins, saponins and lignins (Zachariah et al. 2012). Isoflavones, retinoids, dehydrorotenoids and polyphenolic amides have been suggested to be responsible for the antiviral, antibacterial and antifungal activities of the plant (Serge et al. 2007). Mohammed (2012) and Zachariah et al. (2012) have separately reported that the aqueous and alcoholic extract of *M. jalapa* leaf contains glycosides, tannins, phenolic compounds, resins, alkaloids, proteins, K (161.2 µg/mL), Na (19 µg/mL), Fe (18.7 µg/mL), Zn (14.2 µg/mL), Ca (12 µg/mL), Cd (0.8 µg/mL), Cu (0.3 µg/mL) and Pb (0.1 µg/mL). Column chromatographic analysis of the root of *M. jalapa* revealed the presence of 11 compounds among which were astragaloside II, IV, VI, flazin, 4'-hydroxy-2, 3-dihydroflavone 7-beta-d-glucopyranoside and beta-sitosterol, among others (Lai et al. 2008). Pharmacological studies on *M. jalapa* reported in the open scientific literature included the hepatoprotective effect of the ethanolic leaf extract at 250 and 500 mg/kg body weight (Basini, Mohanalakshmi & Anitha 2013), insulin sensitivity, hypoglycemic and hypolipidemic activities of the root extract in mice (Zhou et al. 2012), antinociceptive activity of the leaf ethyl acetate fraction (Walker et al. 2013), anti-arthritic activity of the hydroethanolic extract of the flower (Augustine et al. 2013), antidiabetic activity of the hydroethanolic extract of the leaf in streptozotocin-induced diabetic rats (Victor, Sowndarya & Moorthi 2015) and antioxidant, free radical scavenging and antimicrobial activities of the leaf (Oladunmoye 2012). The plant has also been reported to be effective in the bioremediation of heavy metal contaminated soils (Yu & Zhou 2009). Proteins from *M. jalapa* have been implicated to possess potent anticancer activity via the apoptotic pathway (Rith et al. 2010). The ethanolic extract of *M. jalapa* leaf has been reported to exhibit nephroprotective and antioxidant activities against acetaminophen-induced renal damage in rats (Shaik, Dhanalakshmi & Jayasree 2012).

Although Nath et al. (2010) and Sharmila-Shaik, Rajendra & Jaya (2012) have separately reported that the leaves and stems are used as a diuretic and tonic, there is still an information gap on scientific data that substantiated the use of the plant as a diuretic. Therefore, the present study was investigated with the aim of evaluating the diuretic potentials of the ethanolic extract of *M. jalapa* leaf in male Wistar rats. An attempt was also made to compare the results with a commonly used reference diuretic drug, furosemide. The choice of ethanol as the extractant in the present study was

based on the need to simulate the traditional method of preparing the extract because it is normally soaked in alcoholic drinks and more importantly for the results of the study to be meaningful and have relevance to the consumers that are largely rural dwellers.

Materials and methods

Plant material

Fresh plant samples were collected from a farmland at Alanamu area in Ilorin, Kwara State, Nigeria. A representative of the sample was authenticated at the herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria, where a voucher specimen was deposited under UIH001/1164 for future reference.

Experimental animals and housing

Thirty male albino rats (*Rattus norvegicus*) with an average weight of 231.50 ± 13.51 g were kept in the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in standard, plastic metabolic cages and acclimatised for 2 weeks before the commencement of the experiment. The male rats were maintained under standard housing conditions (temperature: $28^\circ\text{C} \pm 3^\circ\text{C}$; photoperiod 12 h day/night) and allowed unrestricted access to rat chow (Premier Feed Mill Co. Ltd, Ibadan, Nigeria) and water. The animals were also kept away from the normal movement of students and noise to avoid stress and other physiological effects that may influence diuresis (Asif et al. 2015).

Drugs, assay kits and reagents

Furosemide was a product of Yanzhous Xier Kangtai Pharmaceutical Ltd, China. Assay kits for Na^+ , K^+ and Cl^- were manufactured by Randox Laboratories Limited, County Antrim, United Kingdom. Other chemicals and reagents were obtained from British Drug Houses Laboratory Supplies, Poole, England.

Preparation of ethanol extract of *Mirabilis jalapa* leaf

The fresh leaf of *M. jalapa* was rinsed free of sand and air-dried at room temperature (28°C) for 2 weeks. The dried leaf was pulverised with a domestic blender (Euro premium Mixer Grinder, India). The pulverised leaves (500 g) were extracted in 2 L of 80% ethanol for 72 h at 28°C . The resulting extract was initially filtered with Muslin cloth before using Whatman filter paper No. 1 (Maidstone, United Kingdom).

The filtrate was concentrated *in vacuo* at 45°C using a rotary evaporator to give a greenish black residue of 41.70 g that corresponded to a yield percent of 8.34%. This was reconstituted in 0.9% (w/v) sodium chloride solution (physiological saline) to give the doses of 200, 400 and 600 mg/kg body weight of ethanol extract of *Mirabilis jalapa* leaf (EEMJL) used in the present study.

Grouping and treatment of experimental animals

The procedure described by Lipschitz, Haddian and Kepscar (1943) was adopted for the assessment of diuretic activity of the ethanol extract of *M. jalapa* leaf. Briefly, prior to the commencement of administration, the bladder of the rats was emptied by gentle compression of the pelvic area and pulling of the tails. Thirty male rats that initially received physiological saline solution orally, at 0.05 mL/g body weight (to ensure a uniform water and salt load), were then fasted (without food, but water) for 18 h. These animals were thereafter completely randomised into five groups (A–E) of six animals each and treated as follows:

- Group A (negative control) was administered 1 mL of physiological saline.
- Group B (positive control) received 1 mL that was equivalent to 100 mg/kg body weight of furosemide.
- Group C was administered 1 mL equivalent to 200 mg/kg body weight of EEMJL.
- Group D received 1 mL equivalent to 400 mg/kg body weight of EEMJL.
- Group E was administered 1 mL equivalent to 600 mg/kg body weight of EEMJL.

The administration was done orally and at the same point in time: 10:00 h. Immediately after administration, the animals were individually placed in a metabolic cage (one per cage), kept at room temperature throughout the experimental period. During this period, the animals were placed on restricted access to food and water.

After the administration, the animals were observed closely for 2 h, and then at intervals of 30 min for 5 h duration for any visible sign of clinical toxicity such as salivation, lachrymation, squinted eyes, writhing, convulsion, tremors, yellowing and loss of fur; stress that included excretion of fur and exophthalmia; behaviour abnormalities that included impairment of spontaneous movement, climbing, cleaning of face and ataxia; aggressive behaviour including biting and scratching, licking of tail, paw and penis, intense grooming behaviour and vocalisation; diarrhoea; and mortality. The experimental procedure, including handling of the animals, was carried out according to the Guidelines for the Care and Use of Laboratory Animals of the National Research Council (NRC 2011) and those of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria (BCH/UIIL/08/2012).

Collection of urine and determination of urinary and diuretic parameters

Urine was collected from the animals into graduated vials on an hourly basis for 5 h post-treatment. The volume of urine at each hour was noted and thereafter pooled together for each experimental group after the 5 h observatory period. The urine samples were filtered to remove debris and shedding (Danamma, Jayasimha & Basha 2011; Sathianarayanan et al. 2011). The body weight was

determined by weighing the animals before the 18 h of fasting, after fasting and after treatment using the OHAUS Analytical Balance (OHAUS Corporation, New Jersey, United State of America). The urinary and diuretic parameters that were determined and/or computed included latency of urination, urine pH (using a calibrated pH meter, PHS-25; Shanghai Medic Industry Co., Ltd, Shanghai, China), concentrations of Na^+ , K^+ and Cl^- using a flame photometric procedure described by Tietz (1995), saluretic activity ($\text{Na}^+ + \text{Cl}^-$), natriuretic activity (Na^+/K^+), carbonic anhydrase inhibitory activity, CAI ($\text{Cl}^-/\text{Na}^+ + \text{K}^+$), carbonic anhydrase inhibition index (CAI activity in test group/CAI activity in negative control group), saluretic index (saluretic activity in test group/saluretic activity in negative control group), natriuretic index (natriuretic activity in test group/natriuretic activity in negative control group), diuretic index or diuretic action (urine volume of test group/urine volume of negative control group), Na^+ index (Na^+ excretion in test group/ Na^+ excretion in negative control group), K^+ index (K^+ excretion in test group/ K^+ excretion in negative control group), Cl^- index (Cl^- excretion in test group/ Cl^- excretion in negative control group), kaliuretic index (concentration of K^+ in the urine of test group/concentration of K^+ in the urine of the negative control group), percentage of saline load excreted (volume of urine/volume of saline load [12 mL] \times 100) and diuretic activity or Lipschitz value (diuretic action of the extract/diuretic action of the reference drug) (Asif et al. 2015; Ntchapada et al. 2014; Vogel et al. 2008).

Data analysis

Experimental data were presented as a mean \pm SEM, $n = 6$. One-way analysis of variance was used to compare variables among the different groups. The level of significance among the various treatments was determined by Duncan's multiple range test. The values were considered statistically significant at $p < 0.05$.

Results

Clinical signs of toxicity

Cage-side observation revealed that salivation, lachrymation, squinted eyes, writhing, convulsion, tremors, yellowing and loss of fur were absent in the animals following treatment with the EEMJL and furosemide. In addition, excretion of fur, exophthalmia, impairment of spontaneous movement, cleaning of face, ataxia, biting, scratching, licking of tail, paw and penis, diarrhoea and mortality were not observed at any time during the exposure period.

Effects of ethanol extract of *Mirabilis jalapa* leaf on body weight of male rats

The body weight of the male rats decreased significantly ($p < 0.05$) after fasting for 18 h when compared to their corresponding body weight before fasting. This body weight decreased further after the end of all the treatment except the

animals that received 200 mg/kg body weight of the extract where the decrease in the body weight after 18 h of fasting was not significantly different ($p > 0.05$) from the body weight after the treatment (Table 1).

Effects of ethanol extract of *Mirabilis jalapa* leaf on diuretic parameters of male rats

The ethanol extract of *M. jalapa* leaf significantly increased ($p < 0.05$) the urine volume and the levels of Na^+ and K^+ . The Cl^- ions also increased in the animals that received 400 mg/kg and 600 mg/kg body weight of EEMJL, whereas the 200 mg/kg body weight reduced it significantly ($p < 0.05$). The increases were, however, highest in the animals that received furosemide (Table 2). The latency of urination was significantly shortened after the administration of EEMJL, whereas the pH of the urine increased significantly (Table 2).

The significant increase ($p < 0.05$) in saluretic activity after the administration of all the doses of EEMJL was accompanied by a similar increase in the saluretic index in

a manner similar to those animals administered the reference drug, furosemide (Table 3). Although administration of EEMJL significantly decreased ($p < 0.05$) the natriuretic activity, the carbonic anhydrase activity was reduced by all the doses of EEMJL except that of the 400 mg/kg body weight.

Both the natriuretic and carbonic anhydrase inhibitory activities were reduced by both the EEMJL and the furosemide (Table 3). The natriuretic index and CAI index of the extract-treated animals were lower than that of the physiological saline-administered animals, whereas the diuretic index (diuretic action), kaliuretic index, Na^+ index, K^+ index and Cl^- index were higher than the physiological saline-treated animals. All these changes were similar to those obtained in rats treated with furosemide (Table 3). The percentage saline load excreted and Lipschitz value were higher in the EEMJL-treated animals (Table 4). The furosemide produced the highest percentage saline load excreted, whereas the 400 and 600 mg/kg body weight of EEMJL produced the highest Lipschitz value (Table 4).

TABLE 1: Body weight of male rats after fasting and administration of ethanol extract of *Mirabilis jalapa* leaf.

Treatments	Body weight before fasting (g)	Body weight after 18 h fasting (g)	Body weight after treatment (g)
Physiological saline	209.33 \pm 2.09	198.67 \pm 3.48 ^a (5.09%)	197.67 \pm 1.85 ^a (5.58%)
Furosemide (100 mg/kg body weight)	209.33 \pm 2.60	197.33 \pm 2.66 ^a (5.73%)	181.67 \pm 3.17 ^b (13.23%)
EEMJL (200 mg/kg body weight)	206.00 \pm 4.76	199.00 \pm 5.00 ^a (3.40%)	196.67 \pm 3.84 ^a (4.53%)
EEMJL (400 mg/kg body weight)	207.00 \pm 1.00	185.60 \pm 2.34 ^a (10.34%)	174.67 \pm 1.85 ^b (15.62%)
EEMJL (600 mg/kg body weight)	239.67 \pm 2.60	212.67 \pm 3.17 ^a (11.27%)	201.50 \pm 1.50 ^b (15.93%)

EEMJL, ethanol extract of *Mirabilis jalapa* leaf.

Values are means \pm SEM of six determinations. Test values with superscript letters different from the control are significantly different ($p < 0.05$).

Percentage decreases expressed in parentheses were computed in relation to the body weight before fasting.

TABLE 2: Selected urinary parameters of male rats after oral administration of ethanol extract of *Mirabilis jalapa* leaf.

Treatments	Latency of urination (min)	Urine volume (mL)	Urine pH	Na^+ ion (mmol/L)	K^+ ion (mmol/L)	Cl^- ion (mmol/L)
Physiological saline	35.0 \pm 3.0 ^a	0.93 \pm 0.10 ^a	6.2 \pm 0.1 ^a	136.00 \pm 5.72 ^a	10.60 \pm 0.38 ^a	65.35 \pm 0.90 ^a
Furosemide (100 mg/kg body weight)	18.0 \pm 2.0 ^b	6.43 \pm 1.50 ^b	6.5 \pm 0.1 ^b	176.90 \pm 1.80 ^b	18.50 \pm 1.74 ^b	75.60 \pm 0.82 ^b
EEMJL (200 mg/kg body weight)	17.0 \pm 2.0 ^b	5.60 \pm 0.36 ^c	6.6 \pm 0.2 ^b	170.48 \pm 9.09 ^b	15.20 \pm 1.11 ^c	60.96 \pm 0.67 ^c
EEMJL (400 mg/kg body weight)	14.0 \pm 1.0 ^c	6.01 \pm 0.57 ^d	6.6 \pm 0.2 ^b	153.50 \pm 7.00 ^c	15.25 \pm 1.37 ^c	73.00 \pm 0.88 ^d
EEMJL (600 mg/kg body weight)	12.0 \pm 1.0 ^c	6.00 \pm 0.05 ^d	6.7 \pm 0.2 ^b	156.20 \pm 3.33 ^c	29.46 \pm 1.17 ^d	73.40 \pm 0.68 ^d

EEMJL, ethanol extract of *Mirabilis jalapa* leaf.

Values are means \pm SEM of six determinations. Test values with superscript letters different from the control are significantly different ($p < 0.05$).

TABLE 3: Computed urinary indices of male rats after oral administration of ethanol extract of *Mirabilis jalapa* leaf.

Treatments	Saluretic activity ($\text{Na}^+ + \text{Cl}^-$)	Natriuretic activity (Na^+/K^+)	CAI ($\text{Cl}^-/\text{Na}^+ + \text{K}^+$)	Carbonic anhydrase inhibition index	Saluretic index	Kaliuretic index	Natriuretic index	CAI index
Physiological saline	201.35 \pm 6.62 ^a	12.83 \pm 1.89 ^a	0.45 \pm 0.01 ^a	1.00	1.00	1.00	1.00	1.00
Furosemide (100 mg/kg body weight)	252.50 \pm 2.62 ^b	9.56 \pm 1.03 ^b	0.39 \pm 0.02 ^b	0.87	1.25	1.75	0.75	0.87
EEMJL (200 mg/kg body weight)	231.44 \pm 9.76 ^{cd}	11.22 \pm 0.18 ^b	0.33 \pm 0.04 ^c	0.73	1.15	1.43	0.87	0.73
EEMJL (400 mg/kg body weight)	226.50 \pm 7.88 ^d	10.07 \pm 0.11 ^b	0.43 \pm 0.01 ^a	0.96	1.12	1.44	0.78	0.96
EEMJL (600 mg/kg body weight)	229.60 \pm 4.01 ^d	5.30 \pm 0.08 ^c	0.40 \pm 0.01 ^b	0.89	1.14	2.78	0.41	0.89

EEMJL, ethanol extract of *Mirabilis jalapa* leaf.

Values are means \pm SEM of six determinations. Test values with superscript letters different from the control are significantly different ($p < 0.05$).

Saluretic index: Saluretic activity in test group/saluretic activity in control group.

Natriuretic index: Natriuretic activity in test group/natriuretic activity in control group.

CAI (carbonic anhydrase inhibitory activity): CAI activity in test group/CAI activity control group.

Kaliuretic index = Concentration of potassium ions in the urine of test group/concentration of potassium ions in the control group.

Natriuretic index: Natriuretic activity in test group/natriuretic activity in control group.

Diuretic index: Urine volume of test group/urine volume of control group.

TABLE 4: Other computed urinary indices of male rats administered ethanolic extract of *Mirabilis jalapa* leaf.

Treatments	% Saline load excreted	Lipschitz value	Diuretic index/Diuretic action	Na ⁺ index	K ⁺ index	Cl ⁻ index
Physiological saline	7.75	0.14	1.00	1.00	1.00	1.00
Furosemide (100 mg/kg body weight)	53.58	-	6.91	1.30	1.75	1.16
EEMJL (200 mg/kg body weight)	21.77	0.41	2.80	1.25	1.43	0.93
EEMJL (400 mg/kg body weight)	25.08	0.47	3.24	1.13	1.44	1.12
EEMJL (600 mg/kg body weight)	25.00	0.47	3.23	1.15	2.78	1.12

EEMJL, ethanol extract of *Mirabilis jalapa* leaves.

Values are means ± SEM of six determinations. Test values with superscript letters different from the control are significantly different ($p < 0.05$).

% Saline load excreted = volume of urine/volume of saline load (12 mL) × 100.

Diuretic activity = diuretic action of test dose/diuretic action of standard drug.

Lipschitz value or mean urine volume of test drug/mean urine volume of standard drug.

Na⁺ index: Na⁺ excretion in test group/Na⁺ excretion in control group.

K⁺ index: K⁺ excretion in test group/K⁺ excretion in control group.

Cl⁻ index: Cl⁻ excretion in test group/Cl⁻ excretion in control group.

Discussion

Salivation, lachrymation, ptosis, squinted eyes, writhing, convulsion, tremors, yellowing of fur and loss of hair are useful signs of toxicity, while erection of fur and exophthalmia are physical indicator of stress.

Furthermore, behavioural abnormalities are indicated by impairment of spontaneous movement, climbing, cleaning of face, ataxia and other postural changes, while aggressive behaviour is demonstrated in animals by biting and scratching behaviours, licking of tail, paw and penis. The absence of all these aforementioned signs after the administration of EEMJL indicated that the extract did not elicit any visible sign of toxicity, stress, behavioural abnormality and aggressive behaviour. Administration of EEMJL was also not lethal or the doses administered were still within the lethal dose because no death (mortality) of the animals was recorded throughout the exposure period. The finding in this study with respect to these visible signs of toxicity and abnormal behaviour is similar to that previously reported by Asif et al. (2015) after oral administration of the aqueous extract of *Nigella sativa* to albino rats.

According to Mahnaz et al. (2015), medicinal plants can reduce weight of animals through five basic mechanisms: controlling appetite, stimulating thermogenesis and lipid metabolism, inhibiting pancreatic lipase activity, preventing adipogenesis and promoting lipolysis. Although none of these mechanisms was investigated in this study, the reduction in weight after fasting which was extended to the treatment period might be a consequence of one or a combination of these mechanisms. It is also possible that the plant might have an anti-adipogenic effect preventing adipogenesis because of the presence of phenolic compounds as previously reported by Zachariah et al. (2012). The phenolic compounds can also stimulate the breakdown of fat storage via suppressing phosphodiesterase activity, inducing oxidation of fatty acids, activating the β -adrenergic reception pathway and stimulating the secretion of noradrenaline in fat cells (Dallas et al. 2014). The presence of alkaloids and glycoesters might also suppress appetite and induce satiety through modifying gut hormones and cholecystokinin resulting in a reduction in food and energy intake (Geoffrey et al. 2011).

Such weight reduction will, however, be clinically beneficial to the animals because it may not likely predispose them to being overweight and its associated complications like obesity, cardiovascular diseases and sexual dysfunction.

Diuretics are designed to increase the amount of water and salt expelled from the body as urine.

Consequently, by reducing fluid build-up, diuretics also lower blood pressure. The various types of diuretics, the thiazides, loop and potassium-sparing reduce fluid levels in the body in the form of enhanced urination. The thiazides decrease blood pressure at the same time as they remove excess fluid and relax blood vessels, whereas loop diuretics are used in patients with pulmonary oedema, high blood pressure, kidney problems and heart failure. The potassium-sparing diuretics do not reduce blood pressure, but prevent the loss of potassium. In the present study, EEMJL increased the urine output and reduced the urinary concentrations of the electrolytes. The increase in urinary volume, the Lipschitz value which was more than 1 and the enhanced urinary excretion of electrolytes by EEMJL in the present study may be because of the inhibition of the Na⁺ / K⁺ / 2Cl⁻ co-transporter at the level of the thick ascending limb of the Loop of Henle (Meera et al. 2009), a mechanism similar to the saluretic diuretic, furosemide, used in the current study. Such diuretic activity by EEMJL may also have a peripheral and independent renal vascular action (Elhajili et al. 2001). The shortened latency of urination by EEMJL also suggests that the frequency of urination by the animals was enhanced. All these changes did not significantly change the slightly acidic pH of the urine as evidenced from the pH of the urine that was not significantly different despite slight alteration in this study. The findings with respect to the increase in urine volume and electrolyte excretions in this study are similar to those previously reported by Adam et al. (2013) and Fekadu et al. (2017), after the administration of roots of *Carica papaya* and *Ananas comosus* as well as aqueous crude extract and hot tea infusion of *Moringa stenopetala* leaves to rats.

The diuretic property attributed to EEMJL in the present study was also supported by the increased computed saluretic activity as well as reduced natriuretic and carbonic anhydrase inhibitory activities.

Saliuretic activity is an indicator of the amount of Na^+ and K^+ , whereas the ratio of Na^+ and K^+ indicates natriuretic activity. The Na^+/K^+ ratio is gaining wide acceptance, not only as a translatable biomarker of the mineralocorticoid receptor antagonist, but also as a good indicator of natriuretic activity (Eudy et al. 2011).

Furthermore, a Na^+/K^+ ratio greater than 2 shows the ability of a chemical compound to excrete a greater proportion of sodium ions in contrast to potassium ions (Kumar et al. 2010). Therefore, the higher natriuretic activity exhibited by EEMJL at 200 and 400 mg/kg body weight when compared with that of the furosemide-treated animals suggests the EEMJL excreted more sodium ions than potassium ions at these doses. The calculated saliuretic activity ($\text{Na}^+ + \text{Cl}^-$) and natriuretic (Na^+/K^+) of EEMJL which were more than 2.0 indicated favourable natriuretic effects (Vogel et al. 2008). It is also not surprising that EEMJL displayed some inhibitory activity on the carbonic anhydrase at 400 mg/kg and 600 mg/kg body weight because EEMJL also increased the Na^+ , K^+ and Cl^- .

The diuretic index or diuretic action, which is an indicator of diuretic potential of a chemical compound, is considered to be good if the values are greater than 1.50, moderate if between 1.00 and 1.50, mild when it lies between 0.72 and 1.00 and nil if less than 0.72 (Asif et al. 2013). Therefore, because the computed diuretic index of EEMJL was higher than the 1.50 benchmark, it can therefore be inferred that EEMJL, just like furosemide, has good diuretic action. The best of the diuretic action was, however, exhibited by the reference drug. The computed Kaliuretic index, Na^+ index, K^+ index and Cl^- by the EEMJL, which were higher than the physiological saline-treated animals, consistently emphasise the good diuretic activity of the *M. jalapa* leaf. Findings from this study also suggest that the EEMJL exhibited its diuretic activity by inhibiting the tubular reabsorption of water and accompanying anions, as similarly suggested for some plants like *Spergularia purpurea* as well as roots of *C. papaya* and *A. comosus* (Adam et al. 2013; Jouad, Lacaille-Dubois & Eddouks 2001). Perez et al. (2011) have reported that the main active metabolites from medicinal plants that can intervene on diuretic actions are essential oils, flavonoids, saponosids and potassic salts, and a previous report by Zachariah et al. (2012) has also shown that the aqueous and alcoholic extract of *M. jalapa* leaf contained glycosides, tannins, phenolic compounds, resins, alkaloids and proteins. Therefore, the presence of some of these metabolites may act at the glomerulus of the kidney tubule, provoking an increase in renal circulation, which consequently will enhance the glomerular filtration rate, primary urine formation with a final effect that could be an aquaresis. It may also be that the presence of flavonoids in the plant leaf via increasing the activity of prostacyclin synthase may facilitate the release of renal prostaglandin that has been implicated in diuresis (Gasparotto Junior et al. 2009).

Conclusion

Overall, the data emanating from the present study suggest that ethanol extract of *M. jalapa* leaf exhibited diuretic activity in male rats and thus supports the age-long claim of the use of the plant as a diuretic. The presence of secondary metabolites like flavonoids facilitating the release of renal prostaglandin might be responsible for the diuretic action of the plant. The plant may be explored in the development of complementary and alternative diuretics.

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Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

M.T.Y. designed the study and corrected the article. A.M.O. gathered the data and carried out the statistical analysis. L.A.Q. gathered the data and drafted the article. A.O.A. gathered the data and drafted the article. H.O.B.O. corrected and did the critical revision of the article.

Ethical consideration

The experimental procedures including handling of the animals were humanely done according to the Guidelines for the Care and Use of Laboratory Animals of the National Research Council (NRC 2011) and those of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria (BCH/UIIL/08/2012).

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Data availability statement

New data were generated and analysed in this study. Authors may be contacted in this regard.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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