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Research Article

IN VIVO STUDY ON DIURETIC AND LAXATIVE THERAPEUTIC PROPERTIES OF HYDRO-ALCOHOLIC EXTRACT OF *LU HUI* (LILLIACEAE) ON EXPERIMENTAL RODENTS (*RATTUS NORVEGICUS*)

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ABSTRACT

Objective: In furtherance to the rising evidences for therapeutical potential of *Lu Hui* (*Aloe vera*), the existing study was carried out to evaluate the diuretic and laxative activity of its hydro-alcoholic extract on rodents.

Materials and Methods: The hydro-alcoholic extract of leaves of *Lu Hui* (*HALL)* was prepared by using Soxhlet extractor and subjected to analysis by standard preliminary phytochemical tests. Assessment of both the diuretic and laxative activity was carried out using standard methods. Furosemide (20 mg/kg) was functioned as a positive control for diuretic activity, whereas gaviscon (10 mg/kg) worked as a reference drug for laxative activity.

Results: The HALL showed weight diuretic activity and found to be the most potent in increasing the urinary output at 600 mg/kg when the effect was comparable to that of the standard furosemide. Besides, this extract found to be most effective in increasing urinary electrolyte concentration (sodium, potassium, chloride) at both the doses tested. On the other hand, the results for laxative activity exhibited incredible increase of feces output at the dose of 600 mg/kg, and the increase was similar to that of standard drug gaviscon.

Conclusion: Altogether, the above major findings validate and support its folkloric diuretic and purgative use and lend pharmacological credence to the ethnomedical use of this leaves in the traditional system of medicine, stresses further studies to intricate its active constituents, uses and safety.

Keywords: Bioassay, Diuretic, Laxative, Furosemide, Gaviscon, Ethnomedicine.

INTRODUCTION

Diuretics are drugs that upsurge the rate of urine flow and sodium excretion and are used to regulate the volume and/or composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis. Diuretics not only alter the excretion of sodium (Na^+), but also may modify renal handling of other cations (e.g., potassium (K^+), H^+, Ca^{2+}, and Mg^{2+}), anions (e.g., Chloride (Cl^-), HCO_3^-, and $H_2PO_4^-$), and uric acid [1].

Constipation is a vast ubiquitous public health problem, frequently chronic gastrointestinal disorder with a well-recognized propensity to cause discomfort and to affect the quality of life [2]. Constipation intensifies during aging and can be a chronic condition necessitating the use of laxatives over the long-term. Constipation is not only thwarting but can also cause abdominal distension, vomiting, restlessness, gut obstruction, and perforation, and may be associated with aspiration or fatal pulmonary embolism [3]. The management with classic drugs did not cut, in one hand with the inadequate relief of bloating and other symptoms, and with the lack of efficacy in relieving constipation. Until now, half of patients were not satisfied with the effect of laxatives on refining quality of life [4].

Olden Egyptian Papyrus and Mesopotamian clay tablets describe *Lu Hui* as useful in curing infections, treating skin problems and as a laxative [5]. Cleopatra was said to include aloe cream in her beauty regimen [6]. *Lu Hui* was used by Hippocrates and Arab physicians, and was carried to the Western Hemisphere by Spanish explorers. Legend has it that Alexander the great captured the island of Socotra in the Indian Ocean to secure its *Lu Hui* supplies to treat his wounded soldiers [7]. *Lu Hui* is also popular in both Traditional Chinese and Ayurvedic medicine. The Chinese describe *Lu Hui*'s skin and the inner lining of its leaves as a cold, bitter medication, which is downward draining and used to clear constipation due to the accumulation of heat (fire) [8]; the gel is considered cool and moist. In Ayurvedic medicine, the traditional medicine of India, *Lu Hui* is used internally as a laxative, antihelminthic, hemorrhoid remedy, and uterine stimulant (menstrual regulator); it is used topically, often in combination with licorice root, to treat eczema or psoriasis. In Arabian medicine, the fresh gel is rubbed on the forehead as a headache remedy or rubbed on the body to cool it in case of fever, as well as being used for wound healing, conjunctivitis, and as a disinfectant and laxative [9]. The common names of *Lu Hui* include *Aloe vera* (L.) *Burm. f.*, *Aloe capensis*, *Aloe spicata*, *Barbados aloe*, *Cape aloe, Chirukattali, Curacao aloe, Ghai kunwar, Ghikumar, Indian aloes, Kumari, Rokai, Subr, Zanzibar aloe*. The name Aloe is derived from the Arabic word "Alloeh" meaning a shining bitter substance.

Although reasonably a number of scientific investigations have been undertaken to validate the local use of this plant, there seems to be no previous pharmacological or clinical study was carried out to report the diuretic and laxative activity. Hence, the current exploration was intended at to gauge and to provide experimental evidence for its claimed diuretic and laxative activity of hydro-alcoholic extract of leaves of *Lu Hui* (*HALL)* in rats. Furosemide and gaviscon were selected as the reference drugs for diuretic and laxative activities respectively.

MATERIALS AND METHODS

Plant material

The plant *Lu Hui* was collected in and around Kuala Terengganu, Malaysia. The plant leaves were taxonomically authenticated in UniSZA. The leaves of *Lu Hui* was shade dried, cut into thin slices, powdered coarsely (sieve no. 40) and then extracted in a Soxhlet extractor using 70% of methanol as a solvent at 55°C until the extract becomes colorless. The filtrate obtained by vacuum filtration was concentrated to dryness using a vacuum evaporator under controlled temperature (40-50°C). The dried concentrated extract was suspended in saline before administering to the animals [10].

Preliminary secondary metabolite screening

The crude hydro-alcoholic extract of *Lu Hui* was subjected to preliminary qualitative secondary metabolite screening for the identification of major functional groups and various phytochemical constituents such as carbohydrates, glycosides, alkaloids, flavonoids, saponins, tannins, phenolic compounds, terpenoids, steroids, proteins, gums and mucilage using standard tests [11,12].

Experimental animals

The animal experiments were designed and conducted according to the ethical norms approved by Malaysian government and institutional animal ethical committee for the investigation of experimental pain in conscious animals (AUHAEC 78/FOM/2012). Before beginning the experiments, the albino rats were allowed to acclimatize to animal house condition for a period of 1 week. The animals were fed with standard rodent's chow diet and provided water *ad libitum*. After proper acclimatization, the animals were used for the study.

Acute oral toxicity studies

Wistar albino rats (180-220 g) of either sex were used for acute toxicity studies to determine a safe dose as per method described by acute oral toxic class method of Organization of Economic Co-operation and Development according to 423 guidelines [13]. Acute toxicity studies on HALL extract were performed in experimental rats. Graded doses of the *Lu Hui* (100-1000 mg/kg body weight [BWt]) were administered orally, and the animals were observed for 2 weeks following administration. Changes in BWt, food consumption, and preclinical laboratory findings were noted.

Dosage fixation studies were carried out by administering graded doses of *Lu Hui* extract (100-1000 mg/kg BWt) to control and experimental rats for various time periods; it was found that the herbal extract showed minimum and optimum therapeutic efficacy at a concentration of 300 and 600 mg/kg BWt respectively, administered orally for 30 days. Hence, the dosage schedule was fixed as 300 and 600 mg/kg BWt/rat/day for to ascertain the minimal and optimal therapeutic efficacy.

Evaluation of diuretic activity

Diuretic activity was carried out in accordance with slight modification of the earlier method [14,15]. Twenty-four male rats (180-200 g) were randomly allocated into four groups of six each and were fasted and deprived of water for 18 hrs prior to the experiment. The first group (G-I) of animals serving as a control, received normal saline (15 ml/kg, p.o.); the second group (G-II) received furosemide (20 mg/kg, i.p.) in saline; the third (G-III) and fourth (G-IV) groups received the HALL at the doses of 300 mg/kg (p.o.), 600 mg/kg (p.o.) respectively, in normal saline. The last two groups were functioned as test groups. Immediately after administration on weight basis (at the time of dosing), the animals were hydrated with saline (15 ml/kg) and placed in metabolic cages (3/cage), specially designed to separate urine and feces. Cages kept at room temperature (25±0.5°C) throughout the experiment. The supplement of food and water was withdrawn for next 5 hrs. The volume of urine collected was measured at the end of 5 hr treatment and was subjected to analysis.

Urine analysis

The water excretion rate (urine volume), pH and conductivity of urine collected were estimated using pH meter and conductometer. Furthermore, urine was subjected to determine the concentration of Na^+, K^+ by using systronics mediflame-127 flame photometer. For Na^+ and K^+, the flame intensity corresponding to the concentration of stock solution was noted by using appropriate filters and the instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+. The results were tabulated. The concentration of the Na^+ and K^+ was calculated from the graphs and expressed in terms of mEq/L [16]. The Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using three drops of 5% potassium chromate solution as indicator [17].

Evaluation of laxative activity

Laxative activity was carried out according to the method described previously with little modifications [18]. Thirty rats of either sex (180-220 g) were randomly allocated to five groups of six each and were fasted for 12 hrs prior to the experiment, but with water provided *ad libitum*. The first group (G-I) administered with saline (5 mL/kg, p. o.) acted as a control. The second group (G-II) as loperamide (3 mg/kg. BWt in 0.9% NaCl for 3 days) induced constipated control. The third (G-III) and fourth (G-IV) groups received 300 and 600 mg/kg (p.o.) of the HALL extract in normal saline. The fifth group (G-V) received gaviscon (10 mg/kg, p.o), (It increases intestinal motility through release of active anthraquinones into the colon by colonic bacteria) which served as the reference laxative drug. Immediately after administration on weight basis (at the time of dosing), the animals were placed individually in cages lined with clean filter paper, designed to collect feces [19]. The feces production (total number of normal as well as wet feces) was quantified by measuring feces weight in all four groups, was monitored up to 16 hr.

Statistical analysis

The results were expressed as Mean±SEM (n=6), and data were statistically analyzed using one-way ANOVA, followed by Dunnett's multiple comparison's tests using GraphPad Prism Version 5.0 (GraphPad Software, San Diego, CA, USA) and all the results obtained in the study were compared with the vehicle control group. $p<0.05$ were considered as significant. The control group and treated groups were separately analyzed for statistical significance.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of crude HALL extract revealed the presence of secondary metabolites such as alkaloids, carbohydrates, proteins, tannins, phenolic compounds, flavonoids, terpenoids and glycosides and whereas steroids, saponins and gums and mucilage were absent. The results are shown in Table 1.

Acute oral toxicity studies

In acute toxicity study, it was found that the HALL extract induced diuresis, and there were no significant behavioral, neurological changes and no mortality at any of the tested doses till the end of 14 days of observation (data not shown).

Diuretic activity

The HALL extract was evaluated for diuretic activity using the standard method. For the same, it was initially measured the urinary output, pH and conductivity. It was found that the HALL extract significantly increased the urinary output at a higher dose tested (600 mg/kg, p.o.) when compared to that of standard furosemide (20 mg/kg, i.p.) as shown in Table 2. As there was an increase in urinary output in HALL extract treated rats at 600 mg/kg, it was further intended in investigating the effect of HALL extract on electrolytes concentration and their excretion in order to identify its mechanism of diuretic activity. It was found

Table 1: Preliminary secondary metabolites screening of HALL extract

S. no.	Phytochemical tests	Inference
1	Alkaloids	+
2	Carbohydrates	+
3	Proteins	+
4	Tannin's and phenolic compounds	+
5	Flavonoids	+
6	Steroids	–
7	Triterpenoids	+
8	Saponins	–
9	Glycosides	+
10	Gums and mucilage	–

+: Presence, –: Absence of that secondary metabolites.

Table 2: Effect of furosemide and HALL extracts on urinary volume, pH and conductivity in control and treated rats

Treatment	Dose	Urine volume (ml)	pH	Conductivity
G-I	Saline (15 ml/kg) p.o.	3.85±0.13	7.34±0.98	15.56±0.32
G-II	20 mg furosemide/kg, i.p.	8.56±0.21	7.56±0.65	18.54±0.99
G-III	300 mg HALL/kg, p.o.	3.28±0.67	7.65±0.11	17.11±1.60
G-IV	600 mg HALL/kg, p.o.	6.89±0.19	7.43±0.48	17.59±1.00

Values are expressed as the Mean±SEM (n=6), p<0.05 versus vehicle control (one-way ANOVA and Dunnett's t-test).

Table 3: Diuretic activity of HALL extracts in control and treated rats

Treatment	Dose	Concentration if ions (mEq/l)			Saluretic index			Na^+/K^+ ratio
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
G-I	Saline (15 ml/kg) p.o.	24.11±0.36	8.34±0.34	14.28±0.78				2.84
G-II	20 mg/kg, i.p.	53.52±0.29	31.12±0.31	33.52±0.37	2.56	3.33	2.23	1.72
G-III	300 mg/kg	31.37±0.45	14.89±0.12	17.40±0.53	1.75	1.45	1.27	2.16
G-IV	600 mg/kg	43.43±0.89	22.00±0.17	24.82±0.57	1.74	2.33	1.45	1.94

Values are shown as the Mean±SEM (n=6), p<0.05 versus control (one-way ANOVA and Dunnett's t test), Saluretic index='Test mEql-1/Control mEql-1, SEM, Standard error mean.

that the HALL extract was found to produce a significant increase in excretion of Na^+, K^+ and Cl^- ions at the higher dose, (600 mg/kg p.o.) and the results are shown in Table 3. Changes in other parameters such as conductivity and pH were not significant when compared to that of the control group. The saluretic index and Na^+/K^+ were also determined and shown in Table 3.

Amelioration of bowel obstruction

The administration of loperamide expressively reduced the water intake, water content, the number and the weight of the fecal pellets (Table 4). This indicated that the constipation had been induced in the rats. Yet, no significant difference was observed in the feed intake between the control and the constipated animals. While water consumption lessened in the untreated constipated rats, the administration of the HALL extract substantially improved the water intake in constipated rats (Table 5). Over again, there was no significant difference in the feed intake of all the rodents. Similarly, the HALL extract significantly augmented the number, water content, and weight of fecal pellets in the constipated rats in a dosage-dependent manner.

The BWt of the constipated animals were also regularized following the treatment with the extract. Loperamide significantly diminished the gastrointestinal motility in the untreated constipated rats (Fig. 1). Treatment with the HALL extract, however, improved the gastrointestinal movement in a dose-dependent manner which compared favorably well with gaviscon, a standard laxative drug.

Table 4: Effect of administration of loperamide on intake of feed and water, and properties of faeces of the constipated rats

Parameters	Normal control rats	Loperamide administered rats
Feed intake	18.05±0.12	20.76±1.38
Water intake	22.75±1.36	11.74±1.01
Number of fecal pellets	73.89±2.90	28.93±2.22
Water content of fecal pellets	2.98±0.24	1.74±0.25
Weight of fecal pellets	9.06±0.23	5.16±0.27

Data are mean±standard deviation values (n=6), p<0.05 (one-way ANOVA and Dunnett's t-test).

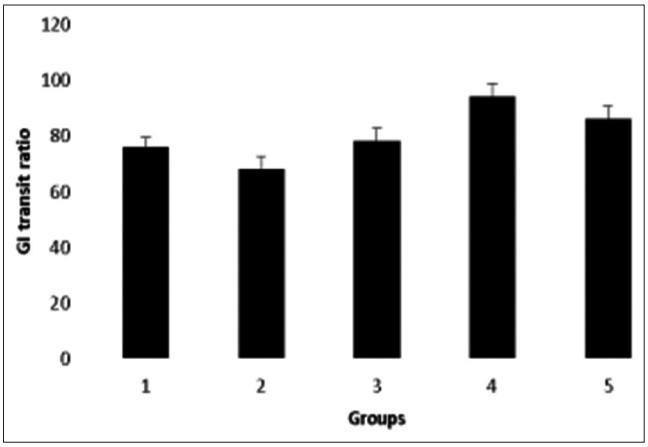

Fig. 1: Effect of HALL extract on the GIT ratio in rats with loperamide induced constipation. Values are expressed as the mean±SEM (n=6), p<0.05 versus vehicle control (one-way ANOVA and Dunnett's t-test)

DISCUSSION

The present research was carried out to evaluate the diuretic and laxative effects of HALL extract as there were no earlier reports supporting its ethnopharmacological folkloric use. In support of earlier reports, the current preliminary phytochemical analysis also revealed the presence of alkaloids, carbohydrates, proteins, tannins, phenolic compounds, flavonoids, terpenoids and glycosides in *Lu Hui* extract (Table 1). Based on earlier investigations and the literature, it was found that *Lu Hui* is regularly consumed, and this herb contains adequate amounts of protein, do not exhibit any toxic properties and even failed to show any toxic effects on brine shrimps [20]. In a continuation to the previous report, the current study on acute oral toxicity analysis also endorsed that HALL extract was safe for administration in rats at the highest dose tested.

The HALL extract was found to be the most potent in increasing the urinary output at 600 mg/kg; the effect was comparable to that of the standard drug, whereas, the extracts at the low concentration (300 mg/kg) was found to be insignificant in increasing the urinary output. The control of plasma Na^+ is important in the regulation of blood volume and pressure; the control of plasma K^+ is required to maintain proper function of cardiac and skeletal muscles. The regulation of Na^+, K^+ balance is also intimately related to renal control of acid-base balance. Determination of urinary electrolyte concentration revealed that, this HALL extract was most effective in increasing urinary electrolyte concentration for all the three ions tested (Na^+, K^+ and Cl^-) at both the doses tested. Cumulatively, these results were also in accordance with the folkloric use of this plant for the treatment of diabetes and hypertension.

The specific conductivity, which is an indirect measure of the ionic content of the urine, was increased in a dose-dependent manner in all

Table 5: Effect of the HALL extract on feed and water intake, body weight gain, and fecal properties of normal and loperamide induced treated rats

	Normal control	Loperamide induced	HALL 300 mg	HALL 600 mg	Gaviscon
Feed intake	30.04±1.55	29.47±2.02	26.67±1.35	27.52±2.03	27.11±0.76
Water intake	57.86±1.26	47.84±1.99	57.99±2.77	59.83±3.01	56.89±2.11
Number of fecal pellets	82.33±1.78	41.65±3.02	67.83±4.04	79.55±3.76	83.66±2.77
Water content of fecal pellets (ml)	2.02±0.51	1.79±0.11	1.99±0.10	2.63±0.09	2.56±0.06
Weight of fecal pellets (gm)	9.45±0.53	5.01±0.31	9.34±0.82	9.87±0.99	10.00±0.41
Body weight gain (g)	14.20±0.69	32.87±0.79	12.21±1.96	11.84±1.55	14.23±1.11

Data are shown as the mean±SEM (n=6), $p<0.05$ versus control (one-way ANOVA and Dunnett's t-test), SEM: Standard error mean.

the extract-treated groups. Thus, the diuretic effect of the extract at both the tested doses is indicated by increase in both water excretion and excretion of Na^+ and K^+. Moreover, the increase in the saluretic index (ratio of concentration of excreted Na^+ and K^+ indicates that the extract increases Na^+ excretion to a greater extent than K^+, which is an essential quality of a good diuretic with minor hyperkalemic side-effect. The K^+ loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally K^+-sparing diuretics are recommended [21].

The active principles responsible for the diuretic effect of the HALL extract have not yet been explicated, but the preliminary secondary metabolite analysis of *Lu Hui* extracts exposed the presence of polar compounds such as flavonoids and terpenoids. Phytochemicals such as flavonoids and terpenoids are known to be responsible for diuretic activity [22]. Hence, the diuretic activity of the HALL extract might be attributed to the presence of these compounds, at least in part by acting synergistically or individually. The current study supports the ethnomedical use of *Lu Hui* as a diuretic agent. Further studies are obligatory to isolate the active constituents and mechanism of action responsible for its diuretic activity.

The use of loperamide as an ameliorative agent for bowel obstruction is well documented. The drug is an opioid agonist antidiarrheal that obstructs intestinal water secretion and colonic peristalsis [23,24]. This reticence extends fecal evacuation time and delays intestinal luminal transit [25]. Loperamide induced constipation is therefore considered to be a model of spastic constipation [26]. The observed decline in the number, weight, and water content of fecal pellets following the treatment with the drug indicated induction of constipation in the rats. A similar observation was reported by Shimotoyodome *et al*. [27] and Wintola *et al*. [28]. The fall in the water consumed by the constipated animals may also be due to the effect of the drug that probably accounted for the drop in the water content of the fecal pellets. However, the drug did not inhibit the animals from feeding adequately. The administration of the HALL extract to the bowel obstructed rats was effective in swaying the increased defecation frequency, fecal volume, and motility of the colon. These are indications of the purgative property of the plant extract. This may be due to the existence of anthranoid glycosides derivatives of which aloin is the main compound [29]. According to Izzo *et al.* [30] aloin is metabolized by the colonic flora to reactive aloe emodin which is responsible for the purgative activity of this plant. This compound possibly exerts its action by disturbing the equilibrium between the absorption of water from the intestinal lumen via an active sodium transport [31] and the secretion of water into the lumen by a prostaglandin-dependent mechanism [32].

Although the feed intake did not differ among the groups, the gain in BWt was higher in the untreated constipated rats compared to the extract-treated groups. This may be due to the accumulation of fecal pellets in their bodies, thus accounting for the extra weight. This clearly indicates that the plant extract increased intestinal secretion and motility in the constipated rats. A similar observation was reported by earlier studies [33,34] where dietary fiber was used for the treatment of morphine-induced constipation in rats. Wintola *et al*. [28] also recorded a similar observation during the treatment of constipated rats with HALL extract. Of particular interest is the fact that the effect of the HALL extract was dose dependent in this study. The effect of the highest dosage actually compared favorably well with gaviscon.

The transit progression of the entire gastrointestinal tract reflected the overall gastrointestinal motor activity. Gauging the colonic transit time is useful in constipation, abdominal bloating, and refractory irritable bowel syndrome [28]. It also provides reckonable information about the colonic transit, enables the identification and characterization of transit abnormalities, and allows the assessment of the severity of the problem as well as the response to therapy [35]. In this study, carmine was used as a marker to measure the colonic movement. The HALL extract augmented the intestinal motility, which in turn, enhanced colonic peristalsis in the rodents. The possible mechanism of the extract in this process may be increasing the release of fluid thereby mounting the intestinal secretion. The laxative effect of the extract could also be accredited to changes in the intestinal motility, which produced an escalation in the intestinal transit and colonic movement [36]. In general, the effect of the treatment with the extract compared favorably well with gaviscon. The existing study compares positively with that of Wintola *et al*. [28] where *Lu Hui* extract was used. This is an indication that this herb was effective in ameliorating bowel obstruction, thereby enhancing easy movement in the intestine.

In summary, preliminary secondary metabolite analysis of HALL extract revealed the presence of alkaloids, carbohydrates, proteins, tannins, phenolic compounds, flavonoids, terpenoids and glycosides. In addition, HALL extract exhibited significant diuretic activity in rats, which may be attributed to the presence of several bioactive diuretic secondary metabolites. The present study further revealed that oral administration of the HALL extract exhibited a purgative activity in rats with loperamide-induced constipation. This suggests the beneficial effects of the HALL extract in improving intestinal motility. Noteworthy is the fact that the HALL extract at the highest dose of 600 mg/kg BWt showed the best laxative action, which compared favorably with gaviscon.

Although an outsized number of active compounds isolated from *Lu Hui*, additional exploration necessary for isolation, structural elucidation, and screening of any of the above revealed active principles to the projected activity of the drug. These studies provide experimental evidence and sustenance to its folkloric diuretic and laxative use and lend pharmacological reliability to the ethnomedical use of this plant in the traditional system of medicine.

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