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Assessment of the diuretic activity of *Hydrocotyle vulgaris* methanolic extract in Albino Mice

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Abstract

This study assessed the diuretic potential of methanolic extract of *Hydrocotyle vulgaris* in albino mice after oral administration. Methanolic extract of *H. vulgaris* (40 and 20mg/kg) and the reference drug, Furosemide (20mg/kg) were administered intraperitoneally using the modified Lipschitz method. All animals were pretreated with saline before commencing the experiment. Their urine output and electrolyte changes were quantitated at several intervals of time after the dose of 5 hours and after 24 hours. The urine output increased significantly in Furosemide and in both 40 and 20mg/kg extracts ($p<0.05$). Methanolic extract of *H. vulgaris* increased the urine volume and electrolyte balance in a dose-dependent manner and qualitatively similar to that of Furosemide. These findings collectively indicate that the extract exhibited significant diuretic activity, providing evidence, at least in part, for its traditional use as a diuretic.

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Introduction

Efficient fluid balance of the body's system helps human beings have a continuous good health. If fluid retention begins to cause health problems, diuretics can help neutralize the fluid imbalance (Jacoby and Youngson, 2005). Diuretics are drugs that increase the rate of water and electrolyte excretion in the form of urine. These are usually prescribed to reduce edema that is linked to heart failure and hepatic cirrhosis (WHO, 2004). Some of these drugs are derived from plants. Examples of plant-derived diuretics are theobromine and theophylline from Theobroma cacao (Taylor, 2000).

The genus *Hydrocotyle* from family Umbelliferae (Apiaceae) is composed of species that are annual and perennial herbaceous of type (Alvarez *et al*., 2008). Only a few species of *Hydrocotyle* make it into attractive aquatics out of the eighty or so that exist. The four most frequent species are so similar that it's difficult to tell them apart without having examples to compare. Among these common species is the *Hydrocotyle vulgaris* (Tor, 2010). *H. vulgaris* is a plant that is indigenous to North Africa and was introduced here in the Philippines just lately (Braker *et al.,* 1995). This plant is common in Malaysia, used by tradition for wound treatment and as diuretic agent (Leong *et al*., 2009). Some of the species of *Hydrocotyle* was already proven to have diuretic property. *Hydrocotyle asiatica* (James and Dubery, 2009) and *Hydrocotyle bonarienses* (Evans, 1992) are among the *Hydrocotyle* species that possess diuretic activity.

Based on research, more and more plants are being proven to increase the formation of urine. *Hydrocotyle asiatica*, also a member of the family Umbelliferae, possesses diuretic properties (Singh *et al*., 2010). Scientific investigations have shown that *H. asiatica's* triterpenic fraction lessened edema and microcirculation alterations (James and Dubery, 2009). Erica multiflora L. of the family Ericaceae and Cynodon dactylon of the family Poaceae are among many plants justified as diuretic agents because both have a notable result on urinary production of water and electrolytes (Sadki *et al*., 2010). *Polygonum barbatum* (L.) Hara var. barbata was also tested for its diuretic property. All extracts of this plant increased urine quantity in a dose-dependent manner particularly the ethyl acetate extract which showed notable distinction of diuresis compared to furosemide (Mazid, 2009).

Since the diuretic activity of *H. vulgaris* has never been experimentally confirmed, the study aims to satisfy these objectives:

1. To assess the diuretic property of *Hydrocotyle vulgaris* methanolic extracts in albino mice and
2. To compare diuretic activity results (urine output and ionic concentration) of the *Hydrocotyle vulgaris* methanolic extracts (20mg/kg and 40mg/kg extracts).

Material and methods

Preparation of Hydrocotyle vulgaris Methanolic Extract

Hydrocotyle vulgaris (Fig. 1) samples were collected from Brgy. Ponong Carigara Leyte, Philippines. The plant was verified and authenticated at Visayas State University, Baybay Leyte. The collected samples were air-dried for two weeks inside the laboratory. Afterward, the samples were powdered coarsely using a blender. Then the powdered samples were sieved and macerated for 15 days with 500ml methanol to 100-gram powdered plant sample ratio. After maceration, samples with methanol were subjected to rotary extraction using a rotary evaporator at 65°C until dried.

Fig. 1. Hydrocotyle vulgaris.

Animals

Adult Swiss albino mice of either sex, weighing 20-25 grams (3-4 weeks old) were obtained from Animal Kingdom Pet Center, Tacloban City. These mice were randomly allocated to four treatment groups (six mice per group) and were housed in standard metal cages (130x130x130mm) designed to separate urine and fecal matter with one mouse per cage. Animals were acclimatized under standard environmental conditions for several weeks in the Chemistry laboratory of the Division of Natural Science and Mathematics in which they had free access to feeds and water ad libitum.

Diuretic activity

The diuretic activity was evaluated following the method of Lipschitz et. Al. with minor modifications. Test animals were deprived of food and water for 18 hours prior to the experiment. After which, each group received a particular treatment. Normal saline of 0.9% NaCl was prepared as one of the treatments. Furosemide was used in the experiment as a positive control. All test samples and positive controls were administered orally by feeding needle. The first group had normal saline (25ml/kg of body weight), the second group had standard diuretic (Furosemide, 20mg/k of body weight) and the last two groups had the methanolic extract of *H. vulgaris* (20mg/kg and 40mg/kg of body weight). Urine was collected in a graduated cylinder every after 1hour for 5hours and after 24hours (no water and food were made available during this period). The diuretic activity was calculated from diuretic action of the animals treated with test samples and positive controls whereas the diuretic action was measured as the quotient between urinary excretion by the test animals treated with test samples and by the control animals (Vogel, 2006). Diuretic activity was calculated as follows:

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test drug}}{\text{Urinary excretion in control}}$$

Urine Output Measurement

Other parameters for diuretic activity were taken into account. The obtained values of pH of the urine output were measured through Ajax Finechem on fresh urine samples. Sodium ion and Potassium ion concentration was measured using the methods of C.G. Garratt (1902), where 0.5ml of urine was measured and 0.25ml of distilled water was mixed in a vial. About 0.007g of dry $CaSO_4$, a little phenolphthalein and dry CaO_2H_2 were added with shaking until the red color was permanent. Then about 0.002g of CaO_2H_2 was further added, mixed, and placed in a water bath at 55° after corking with rubber and was left for 15 minutes.

The mixture was removed from the bath and was left overnight in the cold. Subsequently, the fluid was poured through a filter paper into a centrifugal tube. 0.01g of $(NH_4)_2CO_3$ and a drop of strong NH_3 solution were added. The mixture was mixed well, left for a little time, and filtered through a filter paper into an evaporating dish. The fluid was evaporated to dryness with 0.03g $(NH_4)2SO_4$. The dried mixture was ignited cautiously to gray ash and was moistened freely with H_2SO_4 and was ignited until constant weight over an alcohol lamp. 0.5g Na_2SO_4 was added to get an easier constant weight. The sulphates were weighed and a drop of HCl was added to it. The sulphates were washed out with hot water into a 50ml beaker. It was precipitated at near boiling point and with double normal BaCl. The mixture was left all night undercover and it was poured again into a filter paper with hot water. Dry ignite was collected and the $BaSO_4$ was weighed. From the total sulphates, 0.001 was subtracted for $CaSO_4$ which represented X. From the $BaSO_4$, 0.0025 was subtracted for Y. Sodium and Potassium concentrations were computed as follows:

$$\{(Y \times 0.476) - X\} \times 4.4174 = Na_2SO_4$$

$$X - Na_2SO_4 = K_2SO_4$$

Data Analysis

Experimental values were expressed as mean ± SEM (Standard Error of Mean) of 6 mice. An independent sample student's t-test was carried out for statistical comparison using ANOVA. Statistical significance was considered by a p-value <0.05 as an indication in all cases. Data Analysis was validated using SPSS (version 12.0).

Results and discussion

Hydrocotyle vulgaris is a plant common in Malaysia. It is used traditionally for wound treatment and diuretic agents (Leong *et al*., 2009). This plant was used also to cure whooping cough in Danish folk medicine (Burton, 2002). Since this plant was just recently introduced in the Philippines, many theories about the folklore uses of this plant have been widespread, one of which is its diuretic potential. In this study, methanol was used as the solvent for the crude extract of *H. vulgaris*.

The solvent was evaporated completely to yield a solid mass using a rotary evaporator at 60° to avoid any solvent effect on the experimental mice. The whole plant of *H. vulgaris* was used in the extraction and yielded a dark green, aromatic, oily solid extract.

Oral route was chosen to administer the treatments to meet the way used by people in traditional medicine (Hullatti *et al*., 2011). The diuretic action induced by the methanolic plant extracts was investigated and compared with a standard reference drug, Furosemide, and a control group of normal saline treatment. The urine volumes over the period othe f first 5 hours were measured for the extracts (40mg/kg and 20mg/kg) of *H vulgaris*, Furosemide and normal saline. Furosemide, 40mg/kg extract and 20mg/kg extract of *H. vulgaris* increased the urine flow significantly for the 1st 5 hours ($p<0.05$) when compared to control (Fig. 2). Extract of *H. vulgaris* increased urine output in a dose-dependent manner. From the result, it appears that *H. vulgaris* extracts exhibited diuretic activity at both doses like Furosemide for the first 5 hours but the effect was about 0.25 less than that of Furosemide.

The diuretic effect of the methanolic *H. vulgaris* extract at both dosage (40mg/kg and 20mg/kg) was also significant after 24 hours of observation (Fig. 2). However, there is a slight delayed diuretic activity at the 24th hour. Even though the diuretic activity at 24th hour at both doses were significant, it showed that the extracts acted in a time and dose-dependent manner which could have been a result of the absorption of the active principles in the crude extracts or the extracts could have been stimulating in vivo a diuretic compound.

Table 1. Effect of methanolic extracts of *H. vulgaris* on urine excretion and ionic concentration in albino mice.

Treatment	Cumulative Urine Volume (after 24 hours)	Diuretic Action	Electrolyte Concentration (x100)		
			Sodium	Potassium	Na/K
Normal Saline	0.4 ± 0.02	-	25.72 ± 0.66	22.31 ± 0.58	1.15
Furosemide	1.1 ± 0.17*	2.75	74.24 ± 0.92*	42.13 ± 0.81*	1.76
40mg/kg Extract	0.9 ± 0.13*	2.25	68.97 ± 1.63*	39.6 ± 0.44*	1.74
20mg/kg Extract	0.78 ± 0.11*	1.95	32.92 ± 0.91*	24.90 ± 0.34	1.32

Values are mean ± SEM, n=6, *P< 0.0

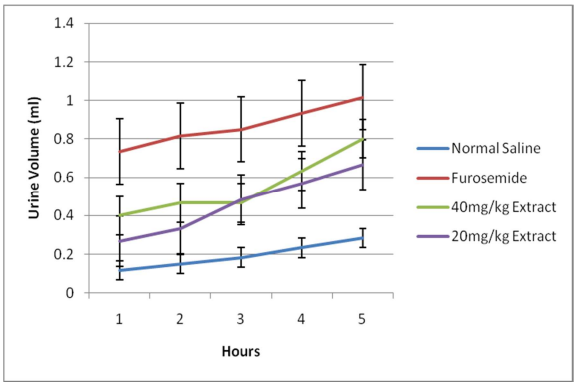

Fig. 2. Time course of diuresis in albino mice with methanolic extracts of *H. vulgaris*, Furosemide and normal saline.

The diuretic effect of a particular extract is indicated by increase in both water and electrolyte excretion (Patel *et al*., 2009). Both dose of the methanol extract induced significant increase in Na+ in the urine, accompanied by a significant excretion of K+ (Table 1). This is a characteristic of a high ceiling diuretic. A significant increase in urinary excretion of Sodium and Potassium suggests that plant extract is acting similarly with furosemide. Furosemide acts by inhibiting electrolyte reabsorption in the thick, ascending loop of Henle causing a profound increase in the output of urine (Shinkawa *et al*., 1993).

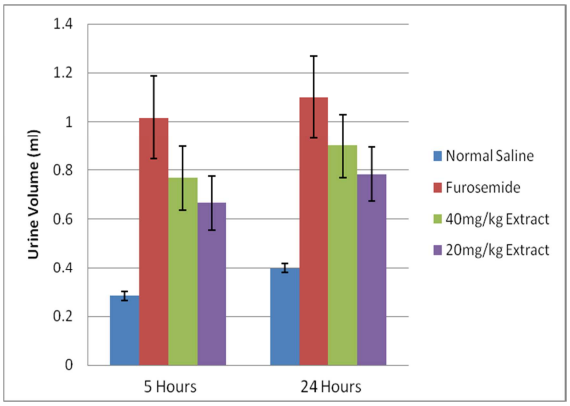

Fig. 3. Urine volume of albino mice treated with methanol extracts of *H. vulgaris*, Furosemide and normal saline in albino mice at 5th and 24th hour by intraperitoneal administration.

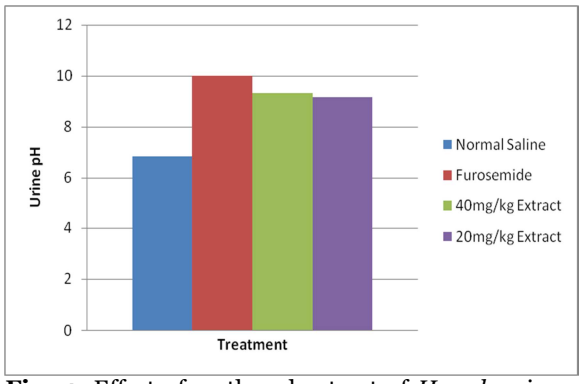

Fig. 4. Effect of methanol extract of *H. vulgaris* on pH in albino mice by intraperitonealadministration.

The increase in the ratio of the concentration of excreted sodium and potassium ions indicates that the extract increases sodium-ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic (Kane *et al.*,2009). The urine pH after administration of *H. vulgaris* extract for both dosages (40mg/kg and 20mg/kg) were 9.33±0.21 and 9.17±0.48 respectively at the 24th hour. Furosemide increased the urine pH by 9.83±0.15 compared to control, thus making the urine more alkaline. All the values were comparable with that of the control, 6.83±0.3 at the 24th hour. This agrees with the observation of Garcia *et al.* (1999) that diuretics make the urine more alkaline. Many studies have shown that the diuretic activity of a plant may be due to the presence of the salts of potassium (Sangita *et al.*, 2009). However, some authors (Leong *et al.*, 2009) have observed that *H. vulgaris* contains flavonoid compounds and saponins and these compounds may be the one responsible for its diuretic capabilities similar to other diuretic herbs that contain flavonoid compounds and saponins.

Conclusion and recommendation

The data reported in this study indicates that *Hydrocotyle vulgaris* methanolic extract showed good diuretic activity, in comparison with Furosemide which is a high ceiling diuretic agent. It was observed that 40mg/kg plant extract showed strong diuretic activity because of more urine output and higher ionic concentration compared to 20mg/kg which suggests that *H. vulgaris* methanolic extract's diuretic activity is dose-dependent. The results showed that the 40mg/kg plant extract of *H. vulgaris* had a similar diuretic spectrum to that of Furosemide. Although it was observed that the plant extract exhibited diuretic activity, further research is suggested to find out the active principles responsible for the diuretic activity.

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