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Full Length Research Paper

An *in vivo* study on the diuretic activity of *Holarrhena antidysenterica*

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Holarrhena antidysenterica is used as diuretic in traditional medicine. The aim of this study was to evaluate the crude extract of *H. antidysenterica* seeds (Ha.Cr) and its fractions, n-hexane (Ha.Hx), n-butanol (Ha.Bu) and aqueous (Ha.Aq), for their diuretic effect in Wistar rats and to investigate whether the activity is concentrated in any of the fractions. Wistar rats kept on fasting for 24 h with water *ad libitum*, divided into normal, positive control and treated groups were orally given normal saline (20 ml/kg), hydrochlorothiazide (HCT; 10 mg/kg) and different doses of the plant material, respectively. Immediately after dosing, the rats were housed in the metabolic cages. The urine was collected at 2 h interval for 6 h and volume, pH and electrolytes levels were measured. Ha.Cr caused dose-dependent (30 and 100 mg/kg) increase in urine output, indicating the diuretic effect. In addition, Ha.Cr increased urine contents of Na⁺ and K⁺, suggesting that the diuretic effect is mediated through increased electrolyte excretion. Similarly, the reference drug, HCT (10 mg/kg), increased urine volume and Na⁺ and K⁺ excretion. None of the resultant fractions exhibited diuretic effect comparable to that of the parent crude extract. Ha.Hx was devoid of diuretic effect, Ha.Bu exhibited a mild diuretic effect at 30 mg/kg, whereas, Ha.Aq caused a significant increase in urine output only at 100 mg/kg, indicating that the diuretic activity is distributed among fractions but in an order of increasing polarity of the solvent. The enhanced diuretic effect in the crude extract as compared to any individual fraction is suggestive of the existence of additive and/or synergistic effect in the crude extract. This study shows the presence of diuretic activity in the *H. antidysenterica* possibly mediated through its saluretic effect, which rationalizes its medicinal use as diuretic.

Key words: *Holarrhena antidysenterica*, diuretic, rats, Na⁺, K⁺.

INTRODUCTION

Diuretics are drugs that promote the rate of urine flow and sodium excretion. Diuretics alone or in combination with other drugs are used in a variety of clinical situations like hypertension, heart and renal failure, nephritic syndrome and cirrhosis (Jackson, 2006). Along with this,

diuretics can also be helpful in diluting the ion contents of the urine, leading to reduction in the supersaturation of stone forming ions and also help in expulsion of crystals, thus preventing recurrent renal stones (Ulmann et al., 1984). The two commonly used diuretics, that is, thiazides and furosemide, have been associated with many side effects, like disturbances of electrolytes, acid-base and water balance, changes in uric-acid, carbohydrate and lipid metabolism and drug interactions (Losse et al., 1983; Ide and Sunagawa, 2007). Therefore,

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there is a need to look for safe diuretics. Herbal medicines are considered to be more safe and economical sources of drugs and also contain synergistic and/or side effects neutralizing potential (Gilani and Attar-Rahman, 2005). Therefore, herbal diuretics can be considered as better therapeutic option, because of their relatively safer and milder actions as compared to diuretics used nowadays which produce several adverse effects due to their strong saluretic effects. As most of the plants contain potassium, along with other nutritional elements like Na^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , etc (Jan, 2011), it can be assumed that they would not lead to potassium depletion (Meléndez, 2004; Gasparotto et al., 2009). *Holarrhena antidysenterica* Wall, commonly known as bitter oleander and locally as “Inderjo Tulkh” or “Kurchi” belongs to the family of Apocynaceae. It is a small deciduous tree found at Himalayan and sub-Himalayan tracts (Baquar, 1989). Traditionally, *H. antidysenterica* is used in the treatment of various disorders like colic, diarrhea, dysentery and fever (Bajrai, 2010). It is also used as carminative, astringent, lithontriptic, tonic, aphrodisiac, cardio suppressant, diuretic and antihypertensive (Baquar, 1989; Usmanghani et al., 1997; Duke, 2002).

In our previous study (Gilani et al., 2010), the phytochemical screening of *H. antidysenterica* has shown the presence of alkaloids, coumarins, flavonoids, saponins and tannins, whereas, conessine, ergosterol, holarrhenine, kurchicine, resin and tannin have been reported among the plant constituents (Kapoor, 1990; Duke, 1992).

H. antidysenterica has been studied for antimutagenic (Atal et al., 1986), antibacterial (Ahmad et al., 1998; Aqil and Ahmad, 2007), immuno-modulatory (Aqil et al., 2008), antioxidant (Kalim et al., 2010), antihyperglycemic (Ali et al., 2011), anti-malarial (Verma et al., 2011), spasmolytic and spasmogenic properties (Gilani et al., 2010). Since the scientific data on the traditional use of *H. antidysenterica* as diuretic is sparse, this study was undertaken to investigate the diuretic activity of its crude extract and the resultant fractions using rats as experimental model.

MATERIALS AND METHODS

Plant, preparation of crude extract and fractions

Dried seeds of *H. antidysenterica* were purchased from local herbal store, identified by a taxonomist, Professor Dr. Jhandar Shah, University of Malakand, Chakdara, Khyber Pakhtunkhwa and voucher specimen (HA-SE-01-08-71) was submitted to the herbarium of the Department of Biological and Biomedical Sciences, the Aga Khan University, Karachi. The detail of the 70% aqueous-ethanol crude extract and its fraction preparation is given in our earlier publication of this plant (Gilani et al., 2010). Yield of the crude extract was approximately 18% (w/w), while n-hexane, n-Butanol and aqueous fractions yielded 10, 25 and 65%, respectively.

Chemicals and drugs

The solvents, methanol, n-butanol and n-hexane, for the extraction and fractionation were obtained from Mark, Darmstadt, Germany. Hydrochlorothiazide (HCT); reference diuretic drug, was obtained from the Sigma Chemicals Co, St Louise, MO, USA. All drugs and chemical used were of analytical grade.

Animals

Wistar rats (180 to 220 g) used for this study were sourced locally and housed at the animal house of the Aga Khan University, kept in plastic cages ($47 \times 34 \times 18 \text{ cm}^3$) with saw dust (renewed after every 48 h), under a controlled temperature of 23 to 25°C and 12 h light-dark cycle. Animals were given standard diet consisting of flour (5 kg), bran (5 kg), molasses (150 g), salt (75 g), nutritive L (33 g), potassium meta bisulphate (15 g), oil (500 g), fish meal (2.25 kg) and powdered milk (2 kg) for a total of 13 kg of the food material. Animals had access to food and water *ad-libitum* throughout the study except 24 h before and during 6 h of diuretic study. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996).

Determination of diuretic activity

Diuretic activity of the crude extract (Ha.Cr) and its fractions, n-hexane (Ha.Hx), n-butanol (Ha.Bu) and aqueous (Ha.Aq), was studied on Wistar rats (180 to 220 g), as described previously (Consolini et al., 1999). Animals were divided with matched body weight and sex into groups of 6 animals each. Normal saline and positive control groups received oral doses of saline (20 ml/kg) and standard diuretic drug: HCT, 10 mg/kg body weight, respectively. The rest of the groups were given 2 to 3 different doses of the test material dissolved in saline. Subsequently, the animals were placed individually in metabolic and diuretic cages (Techniplast, 21020 Buguggiate-Va-Italy). The urine was collected in graduated cylinders for 6 h at 2 h intervals. Total urine excreted out was collected and the volume was determined. pH of the pooled urine from each animal was determined by using pH meter (Orion* 320 PerpHecT* Thermo Scientific) and Na^+ and K^+ concentrations was determined on flame photometer (Flame Photometer 410, Corning).

Statistical analysis

The data are expressed as mean \pm standard error of mean (SEM). All statistical comparisons between the groups are made by mean of one-way analysis of variance (ANOVA) with post hoc Dunnett's test, using GraphPad Prism (GraphPad Software, San Diego, CA, USA). P value less than 0.05 is regarded as significant.

RESULTS AND DISCUSSION

In this study, the diuretic activity of the crude extract of *H. antidysenterica* and its fractions were investigated to rationalize its medicinal use as a diuretic agent, which might also be contributing to its antihypertensive and antiurolithic activities. Urine output/100 g body weight/6 h in saline treated group was $0.98 \pm 0.12 \text{ ml}$ (mean \pm SEM), while HCT (10 mg/kg) increased it to $2.68 \pm 0.23 \text{ ml}$ ($P <$

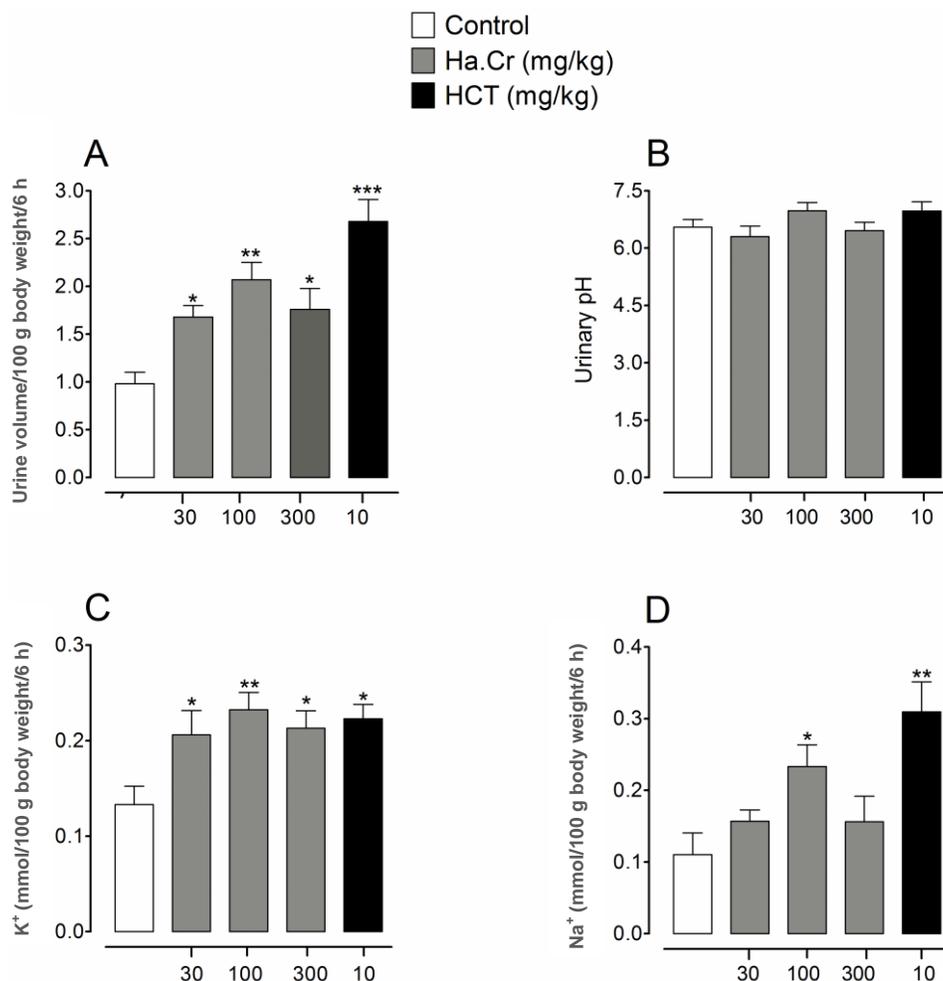


Figure 1. Effect of the crude extract of *H. antidysertrica* and hydrochlorothiazide (HCT) on urine volume (A), pH (B) K⁺ (C) and Na⁺ contents (D), collected after 6 h of their administration (values shown are means \pm SEM, n = 6). *P < 0.05, **P < 0.01 versus saline treated group.

0.001), as shown in Figure 1A. Ha.Cr at the doses of 30, 100 and 300 mg/kg increased the urine volume to 1.68 ± 0.119 ml ($P < 0.05$), 2.07 ± 0.18 ml ($P < 0.01$) and 1.76 ± 0.22 ml ($P < 0.05$), respectively, indicating its diuretic effect (Figure 1A). At the highest dose (300 mg/kg) used in this study, there was blunting of the response by approximately 20% as compared to 100 mg/kg. The maximum diuretic efficacy of Ha.Cr was about 20% less than that of HCT in terms of cumulative urine volume. The decrease in the diuretic activity on higher doses could be due to the co-existence of antidiuretic component(s) in Ha.Cr, as plant extract may exhibit multiple therapeutic activities probably on account of having a mixture of phytochemical. The presence of synergistic and/or side effect neutralizing effect in plants is known to exist, which is probably meant by nature not to allow the pharmacological effect go beyond its therapeutic limit,

above which it could have caused toxic effects (Gilani and Atta-ur-Rahman, 2005). In addition to its effect on urine volume, Ha.Cr also increased urine excretion of Na⁺ and K⁺ (Figure 1C and D). Total K⁺ and Na⁺ contents (mmol/100 g body weight) in 6 h pooled urine samples were 0.21 ± 0.02 ($P < 0.05$) and 0.16 ± 0.01 , 0.23 ± 0.02 ($P < 0.01$) and 0.23 ± 0.03 ($P < 0.05$), and 0.21 ± 0.02 ($P < 0.05$) and 0.16 ± 0.03 ($P < 0.01$), respectively, at 30, 100 and 300 mg/kg, as opposed to, 0.13 ± 0.02 and 0.11 ± 0.03 , with saline group. The reference drug HCT also increased the K⁺ and Na⁺ excretion to 0.22 ± 0.01 ($P < 0.01$) and 0.31 ± 0.04 ($P < 0.01$) mmol/100 g body weight/6 h, respectively (Figure 1 C and D), whereas, the urine pH did not change significantly in any group (Figure 1B). The mechanism of diuretic actions of Ha.Cr is possibly due to the increase of electrolytes, like K⁺ and Na⁺ excretion by inhibiting their tubular reabsorption, a

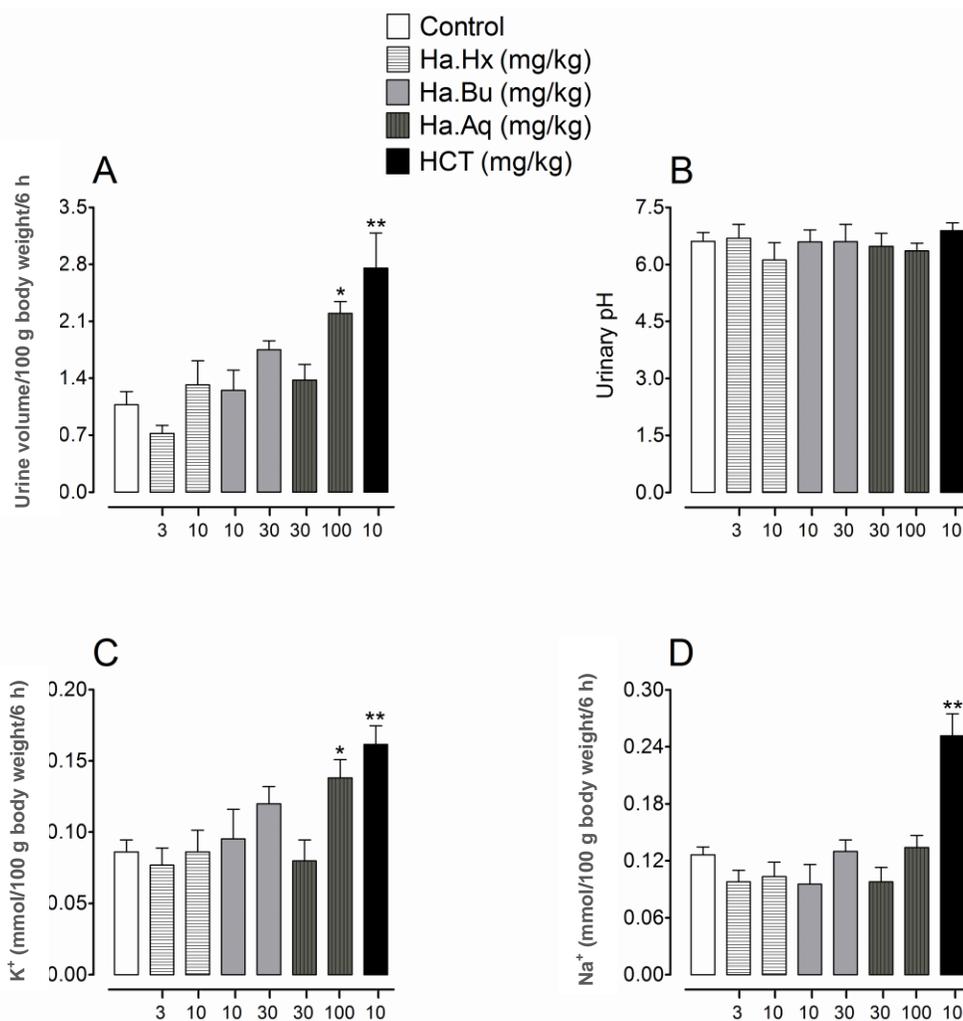


Figure 2. Effect of the n-hexane, n-butanol and aqueous fractions of *H. antidysentrica* extract on urine volume (A), pH (B), K^+ (C) and Na^+ (D) contents, collected after 6 h of their administration. (Values shown are means \pm SEM, n = 6). *P < 0.05, **P < 0.01 versus saline treated group.

a mechanism attributed to the standard drug used in this study (hydrochlorothiazide). The presence of flavonoids and saponins in the plant extract (Gilani et al., 2010) could be contributing in its diuretic effect, as these phytochemicals are known to possess diuretic properties (Maghrani et al., 2005), however, additional mechanism(s) may not be ruled out.

In order to check whether the diuretic effect is concentrated in any of the fractions, we carried out the activity-guided fractionation and evaluated their diuretic effect. The doses of the fractions were selected in proportion to the percentage yield of the fractions, obtained from crude extract. Figure 2A shows the effect of different fractions on the urine output. Ha.Hx was devoid of any diuretic affect, the Ha.Bu showed a mild increase in the urine volume (1.75 ± 0.11 , $P > 0.05$) at the

dose of 30 mg/kg, while the aqueous fraction showed significant increase (1.92 ± 0.24 , $P < 0.05$) only at the dose of 100 mg/kg, as compared to normal saline (1.07 ± 0.16) (Figure 2A). Like crude extract, there was no change observed in pH and Na^+ excretion of all the groups (Figure 2B and D), whereas, urinary excretion of K^+ was slightly increased (0.12 ± 0.012 , $P > 0.05$) by Ha.Bu at the dose of 30 mg/kg and significantly by Ha.Aq (0.14 ± 0.01 , $P < 0.05$) at the dose of 100 mg/kg, as compared to the normal saline group (0.08 ± 0.008), as shown in Figure 2C. Whereas, HCT very significantly increase urinary excretion of both K^+ (0.16 ± 0.01 , $P < 0.01$) and Na^+ (0.25 ± 0.02 , $P < 0.01$) at 10 mg/kg (Figure 2C and D). Loss of efficacy with fractionation suggests the existence of multiple constituents exhibiting additive and/or synergistic effect in the crude extract that is

segregated among the fractions, thereby, reducing intensity of the diuretic action.

Diuretics alone or in combination with other antihypertensive drugs are considered to be more effective than the calcium channel blockers and angiotensin converting enzymes inhibitors as the first line treatment of hypertension. It also helps in the prevention of one or more forms of cardiovascular diseases in high-risk patients with hypertension (Boger-Megiddo et al., 2010). The seventh report guidelines issued in the United States by the Joint National Committee on prevention, evaluation, and treatment of high blood pressure, and England and Wales, the National Institute for Health and Clinical Excellence guidelines recommend the use of low dose diuretics as first line pharmacological treatment for high blood pressure (Boger-Megiddo et al., 2010). The diuretic therapy is also useful in the treatment of edema, hypercalcemia, hepercalceuria, diabetes insipidus and acute renal failure (Krumlovsky and del Greco, 1976). The presence of diuretic activity in *H. antidysenterica* may explain the medicinal use of the plant in hypertension and urolithiasis, though additional mechanism(s) may not be ruled out.

Conclusion

In this study, we found the presence of diuretic effect in *H. antidysenterica*, possibly mediated through its saluritic potential. All the fractionations were found less efficacious than the parent crude extract suggesting the existence of additive and/or synergistic effect in the crude extract. These results provide rational for its medicinal use as a diuretic agent.

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