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An investigation of diuretic activity of aqueous extract of *malus sylvestris* (l) mil fruits in experimental animal models

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Abstract

Objective and background: *Malus sylvestris* Linn are a plant widely used as a traditional medicinal herb has a wide range of Pharmacotherapeutic activities. The current study was carried out to investigate the diuretic activity of *Malus sylvestris* Linn fruit aqueous extract (AEMS) in experimental animals. **Methods:** In the current study Wister rats were divided into 5 groups of n=6 each using Furosemide (100 mg/kg, p.o) as the standard drug and normal saline as the control. AEMS is given at the doses of 200 mg/kg, 400 mg/kg and 800 mg/kg p.o. in distilled water respectively. Lipchitz method was used for his study. Parameters such as volume of urine, concentration of sodium and concentration of potassium are noted. **Results and discussion:** The study revealed that extract of AEMS has considerably increased the urine volume with significant increase in cationic concentration at increased doses. The concentration of Na⁺/K⁺ indicates the dose dependent response with the comparable results at the doses of 200 mg/kg, 400 mg/kg and 800 mg/kg with reference with the standard administered. Oral administration of AEMS at the dose of 800 mg/kg shows high significance (P<0.001) with that of control and dose of AEMS of 400 mg/kg shows significance (P<0.05) shows and dose of AEMS of 200 mg/kg shows less significance (P<0.01) with decreased urine output with that of control. **Conclusion:** As a result of foregoing study aqueous extract of *Malus sylvestris* Linn fruit has considerable diuretic activity at higher doses.

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Introduction

Nature has provided a plethora of remedies to cure all of mankind's ailments. Historically, plants were the ultimate source of almost all medicines, with the plant serving as man's sole chemist for centuries [1]. Diuretics are medications that enhance the amount of water and electrolytes the kidneys excrete in the urine. One or more reabsorptive processes taking place at various nephron segments are the cause of the net loss of salt and water in the urine. Actually, the increased salt chloride excretion is the primary cause of the increased water loss. This is accomplished either directly by affecting certain nephron segments or indirectly by changing the components of the urine filtrate. Diuretics are recommended for a number of ailments, including congestive heart failure, liver cirrhosis, nephrotic syndrome, hypertension, hyperkalaemia, and the treatment of toxic anions intake. Diuretics can cause adverse effects such hyperuricemia, hypercalciuria, hypomagnesaemia, hypokalaemia, ototoxicity, hyperglycaemia, erectile dysfunction, hypersensitivity reactions, and others, just like

other drugs. Therefore, it is urgently necessary to find better medications with fewer side effects that are also more effective and cost-effective. Different medications are derived from plants. They present unrealized potential for more modern medications [2].

Diuretics are those drugs that are used to treat Hypertension and peripheral oedema [3]. Diuretics bring about an increase in urinary volume as well as in electrolyte output [4]. Life-threatening conditions include CHF, Nephritic syndrome, cirrhosis, renal failure, and hypertension can benefit from drug-induced diuresis. The majority of diuretics are risky and have detrimental consequences on health and well-being, including impotence, weariness, and weakness. Na⁺ absorption is inhibited by caffeine, a naturally occurring diuretic found in coffee, tea, and cola. The search for potassium-sparing diuretics began despite the fact that most diuretics have been discovered to be highly effective in increasing salt excretion, they all also result in potassium loss [5].

Malus sylvestris, sometimes known as European Crab apple or common apple, is a member of the Rosaceae family. It is a tiny deciduous tree with a height of 4 to 10 meters. The fruit has a roughly spherical shape, is 2.5 x 2.8 cm, is flattened at each end, and is glossy, pale green with huge white dots that turn flushed or spotted crimson in the fall. The fruit is rich in carbohydrates, total dietary fats, sugars, proteins, fats and minerals. Polyphenols (Tannins, Anthocyanins), Saponins, Alkaloid and Steroid Flavonoids like Procyanidin, quercetin, phloretin, myricetin, and epicatechin are all flavonoids classified under the flavanol (catechin) are present in the studies of the compounds isolated from crab apple. The plants have a variety of pharmacological properties, including nerve sedation, anxiety relief, blood pressure reduction, carminative, digestive, emollient, hypnotic, laxative, refrigerant, antioxidant, and antibacterial properties. In folklore, they are used to treat cancer, malaria, warts, dysentery, fever, scurvy, and spasms. Crushed fruits are used to treat inflammation, minor wounds, and sore throat [1].

In order to reduce the negative effects of synthetic medications and to encourage the use of an innovative natural medicinal plant, the current study was conducted to investigate the diuretic activity of *Malus sylvestris* Linn fruits.

Materials and Methods

The fresh leaves of *Plectranthus scutellarioides* were obtained from a local village in Mangalore and authenticated by botanist, Pilikula Nisargadhama, Vamanjoor, Mangalore. It is preserved in the departmental library for future reference. The current study was carried out at the Karavali College of Pharmacy's Department of Pharmacology.

Animals

The following study used Wistar rats of both sexes weighing around 150-200g. The animals were fed a standard pelleted diet (Lipton India Ltd., Mumbai) and distilled water ad libitum under a constant 12 hours light and dark cycle. Prior to the experiment, all animals were housed in laboratory conditions for 5 days. All experiments were carried out in accordance with the ethical standards for the investigation of experimental pain in animals and the guidelines for the investigation of inflammation in conscious animals.

Plant Extract

The fruits were washed 2 or 3 times with tap water so that it was made free from all dust materials. They were cut into small pieces and made into a paste with the help of a blender. For the aqueous extract, 500 g of plant material was extracted by infusion boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration. The final crude extract was a brownish greasy powder with a dry weight percentage of 15.2% d.w. The extract was stored in refrigerator at 4 – 8 °C [6].

Experimental Design

Acute toxicity studies

Acute toxicity study was performed in accordance with OECD guidelines 425. No adverse effect or mortality was detected in albino rats up to 2 gm/kg, p.o of *Malus sylvestris* during the 24 to 72 hrs observation periods. For this period the rats were continuously observed for 5 hrs for any gross behavioral, neurological or autonomic toxic effect and lethally after 24 to 72 hrs.

Lipchitz method for diuretic activity

Wister rats were divided into 5 groups of n=6 each.

Group I – Received normal saline (25mL/kg) orally.

Group II – Received furosemide (100mg/kg) orally.

Group III – Received 200 mg/kg AEMS fruit orally.

Group IV – Received 400 mg/kg AEMS fruit orally.

Group V – Received 800 mg/kg AEMS fruit orally

Group 1 served as the control (vehicle treated), Group II served as the standard (received Furosemide 100mg/kg body weight), and Groups III, IV, and V were given aqueous extract of *Malus sylvestris* fruit at doses of 200mg/kg, 400 mg/kg, and 800mg/kg body weight, respectively. The rats were placed in metabolic cages specially designed to separate urine and feces immediately after administration and kept at room temperature of 25±0.5°C. Urine was collected in a measuring cylinder for up to 6 hours. Animals were not given food or water during this time. The volume of urine collected for each group was measured. The parameters taken for each individual rat were urine volume (concentrated for water intake during the test period), and concentration of Na⁺ and K⁺ in urine. The content of Na⁺ and K⁺ in the urine was estimated by ICP-OES (inductive coupled plasma – optical emission spectroscopy).

Results and Observation

The studies on diuretic activity revealed that the extract of a *Malus sylvestris* has considerably increased the urine volume with significant increase in the cationic concentration at each increased dose. The concentration of Na⁺/K⁺ indicates a dose dependent response with comparable results at the dose of 200, 400 and 800 mg/kg with that of the reference standard drug administered. The reference diuretic (Furosemide), increased urine volume up to (3.5±0.04ml). Oral administration of AEMS at a dose of 800 mg/kg shows high significance (P<0.001) with increased urine output (Table 2) with that of control AEMS at a dose of 400 mg/kg shows the significance (P<0.01), whereas 200 mg/kg shows less significance (P<0.05) with decreased urine output with that of control.

Table No.1: Effect of aqueous fruit extracts of *Malus sylvestris* on urine volume and Na⁺ and K⁺. Concentration in rats by using Lipchitz diuretic model.

Group	Treatment	Urine Volume ml/kg	Concentration Of Excreted Ions	
			Na+ (mEq/L)	K+ (mEq/L)
I	Normal saline (25ml/kg)	2.53±0.088	60.15±0.309	48.67±0.494
II	Furosemide (100mg/kg)	10.28±0.174***	134.5±1.335	121.2±0.703
III	AEMS-200 mg/kg	3.23±0.164*	66.00±0.577	52.17±0.872
IV	AEMS-400 mg/kg	3.40±0.057**	67.50±0.562	53.00±0.577
V	AEMS-800 mg/kg	6.90±0.189***	104.2±2.136	91.17±0.792

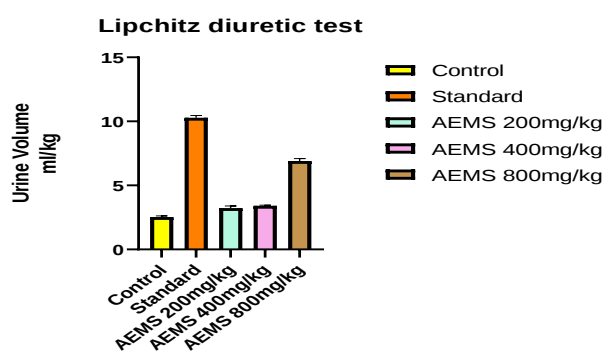


Figure 1. Comparative profile of volume of urine output after oral administration 200, 400, 800mg/kg of AEMS.

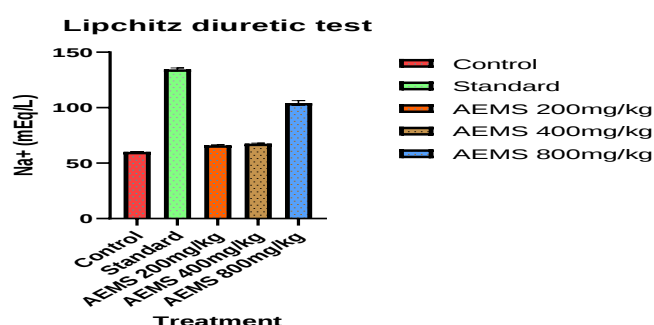


Figure 2. Comparative profile of concentration of Sodium ions after oral administration 200, 400, 800mg/kg of AEMS.

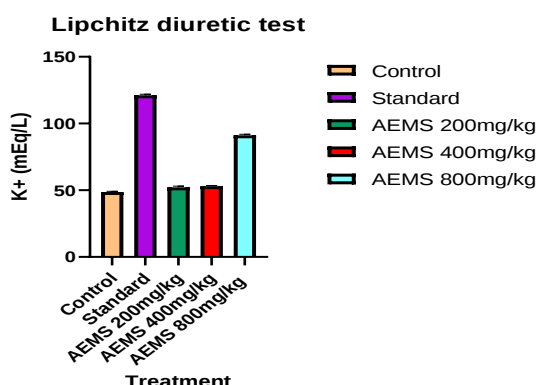


Figure 3. Comparative profile of concentration of Potassium ions after oral administration 200, 400, 800mg/kg of AEMS

Discussion

Diuretics are prescribed to treat both pulmonary congestion and peripheral edema. These medications aid in the treatment of volume overload syndrome, which includes orthopnea and paroxysmal nocturnal dyspnea. They increase plasma volume, which increases venous return to the heart. This lowers blood pressure by reducing cardiac work, oxygen demand, and plasma volume. According to the current study's findings, diuretics play an important role in hypertensive patients [7]. The diuretic activity investigations demonstrated that the extract of *Malus sylvestris* considerably increased urine volume with a considerable increase in cationic content at each higher dose. The Na⁺/K⁺ concentration is dose-dependent, with comparable results at dosages of 200, 400, and 800 mg/kg with the reference standard medication given.

The fruit (*Malus sylvestris*) contains Carbohydrate, Total dietary fiber, Sugar, Polysaccharides, Protein, Fat and Minerals [8].

The presence of potassium salts or components responsible for hypotensive activity may explain the plant's diuretic activity. Further research, such as the separation and characterization of the diuretic component from the plant's aerial portions, is required to confirm the activity.

The current investigation suggests that the fruit of *Malus sylvestris* has diuretic properties.

Conclusion

According to the findings of the study, aqueous extract of *Malus sylvestris* Linn fruit is a possible diuretic. The data was acquired completely using a controlled experiment methodology with lab animals. All of the information gathered has been statistically validated. However, more research is needed to identify the Phyto-constituents responsible for diuretic activity and to establish the safe use in renal diseases.

Conflict of Interest

No conflict of interest.

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