Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 1 |JAN-MAR |2013|98-102

Research Article
Pharmaceutical Sciences

EXPERIMENTAL EVALUATION OF DIURETIC ACTIVITY OF AEGLE MARMELOS IN RATS

[1]Seema Singh*, [2]Sandeep Kumar Singh, [3]Shobit Srivastava, [3]Pramod Singh, [4]Mohit Trivedi, [3]Pratap Shanker, [3]R.K.Dixit, [5]Romi Singh Rana

[1]Department of Pharmacy, Northern India Engineering College, Lucknow, (U.P.)
[2]Department of Pharmaceutical Sciences, Dr. H. S. Gaur Central University, Sagar (M.P.)
[3]Department of Pharmacology, King George Medical University, Lucknow (U.P)
[4]Department of Pharmacology, E.L.M.C. Lucknow, (U.P.)
[5]Department of Urology, King George Medical University, Lucknow (U.P.)
*Corresponding Author Email: seema_b.pharm@rediffmail.com

ABSTRACT

Purpose: The aim of the present study was to investigate the diuretic activity of ethanolic extracts and its fractions of Aegle marmelos fruit in experimental models. Ethanolic extracts and its fractions of Aegle marmelos ripe fruit were administered to experimental rats intraperitonealy at doses of 300, 400 and 500 mg/kg i. p. Control group received only normal saline (25ml/kg) through intraperitoneal route. Standard group received Furosemide (100mg/kg). The diuretic effect of the extracts was evaluated by measuring urine volume and sodium content in urine.

KEY WORDS

Aegle marmelos, diuretic activity

INTRODUCTION

Diuretics are used in many clinical conditions including the edematous disorders and hypertension. Though all diuretics are used to increase renal excretion of sodium and water, they differ considerably in chemical derivation, efficacy, sites and mechanism of action. The choice of a diuretic clinically depend on is the objective of therapy and the pathophysiology of the patient's disease. Patients with renal insufficiency require loop diuretics because they do not respond to other agents to a clinically relevant degree. Patients with cirrhosis are reported for have secondary hyperaldosteronism as a cause of sodium retention and diuretic treatment in such patients is initiated with an inhibitor of aldosterone, spironolactone. Effective use of diuretics requires knowledge of the pharmacology of each diuretic agent coupled with an understanding of the pathophysiology of the patient's disease. Other uses include the treatment of hypertension and the treatment of cerebral edema [1]. A global reliance on alternative system of medicine for chronic and acute ailments resulted in an intense area of research and discovery of a number of herbs with potential to curb diseases. Among them, ample number of herbs has been exploited for modulation of immune system from Ayurvedic formulations either alone or in combinations [2]. Traditionally, various parts of the plant, *A. marmelos* Corr. (Rutaceae) has been in use the treatment of a variety of disorders [3]. The plant is reported to have multiple therapeutic properties such as anti-inflammatory, antipyretic and analgesic [4] anti diabetic [5] anti diarrhoeal

[6] anti hyperlipidemic [7] antifungal [8] antimicrobial, antibacterial, anti parasitic [9] anti cancer [10] insecticidal activity [11] anti malaria [12] hepatoproctective [13] antigenotoxic activity [14] cytoprotective effect [15] immunomodulatory activity [16] anticonvulsant Activity [17] hypolipidemic activity [18] antifertility effect [19] and cardioprotective potentials [20]. However the diuretic activity of *A. marmelos* has more been reported so far. Therefore present study was planned to see the diuretic activity of *A. Marmelos* in animal in animal experimental model.

MATERIALS AND METHODS

Plant collection

Ripe Fruits of *Aegle marmelos* (Bael) were collected in the month of March from local market of Lucknow (Uttar Pradesh). The fruits were authenticated as *Aegle marmelos* (Rutaceae) by pharmacognostic evaluation and a voucher specimen was deposited at Taxonomic Division of National Botanical Research Institute Lucknow, for future reference. (Voucher no. NBRI/CIF/148/2010).

Preparation of ethanolic extract

The plant materials were dried in the shade and size reduction of air-dried ripe fruit pulp of *Aegle marmelos* was done by a mechanical grinder. The powder of *Aegle marmelos* was initially defatted with petroleum benzene (60 - 80˚C) followed by 1000 ml of ethanol, by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using whattman filter paper (No.1) and then concentrated in a vacuum and dried at 45˚C for ethanol elimination and to obtain a dark-brown residue. The extracts were kept in a sterile bottle under refrigeration conditions of about 2-8˚C for further studies.

Experimental animals

Male wistar rats weighing 100-200 gm were obtained from the animal house, faculty of pharmacy, NIEC, Lucknow (U.P.) the animals were housed in polypropylene cage with steel net and maintained under standard conditions of temperature 25±5ºc and 55±5 relative humidity with a regular 12 hours light and 12 hours dark cycles and allowed free access to standard laboratory food and water. All animals are treated humanely in accordance with guidelines from case of animals as set by IAEC.

Diuretic activity

The diuretic activity of ethanolic extracts of *Aegle marmelos*, petroleum ether fraction of *Aegle marmelos*, ethyl acetate fraction of *Aegle marmelos*, chloroform fraction of *Aegle marmelos* and Furosemide was carried out by using *in-vivo*, Lipschitz test method. The rats were divided into 6 groups each containing different no. of animal depending upon dose, at least 3 animals are required for each dosing and deprived of food and water for 18 hours. All the rats received priming dose of normal saline (25ml/kg) orally. Both the extracts and Furosemide (Standard) were dissolved in a normal saline.

- Group I served as control in which only normal saline (25ml/kg) was administered through intraperitoneal route.
- Group II served as standard received Furosemide (100mg/kg).

Rest of the groups served as treated groups.

- Group III received Ethanolic extract of *a. marmelos* at the dose levels of 300, 400 and 500 mg/ kg i.p., respectively.
- Group IV received Petroleum ether extract of *a. marmelos* at the dose levels of 300 and 400 mg/ kg i.p., respectively.
- Group V received Chloroform extract of *a. marmelos* at the dose levels of 300 and 400 mg/ kg i.p., respectively

- Group VI received Ethyl acetate extract of *a. marmelos* at the dose levels of 300 and 400 mg/kg i.p., respectively.

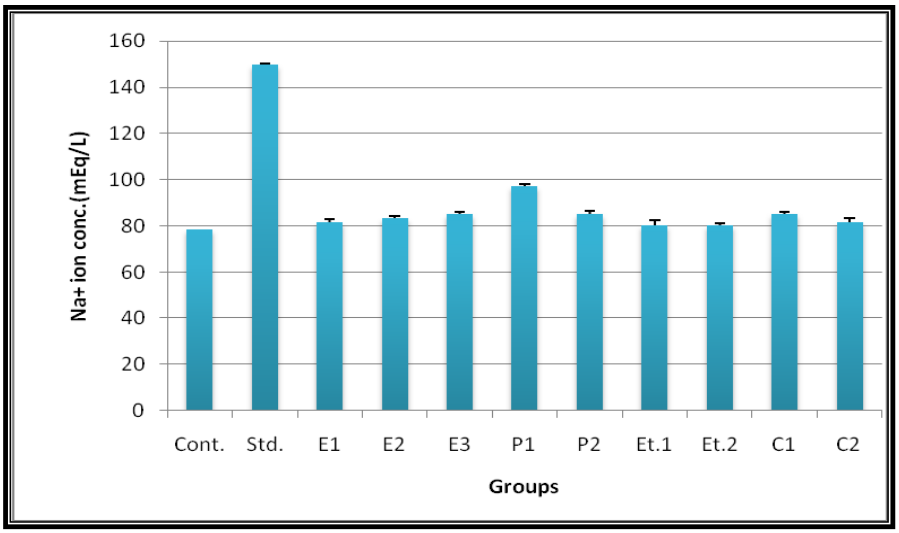
Immediately after administration, the rats (one in each cage) were placed in metabolic cages specially designed to separate urine and faeces and kept at room temperature of 25±0.5ºC. The urine was collected in a measuring cylinder up to 24 hrs. During this period, no food or water was made available to animals. The volume of urine collected was measured for all the groups. The parameters taken for each individual rat were body weight before and after test period, urine volume (concentrated for water intake during the test period), and concentration of Na^+ in urine. The content of Na^+ in the urine was estimated by flame photometry.

STATISTICAL ANALYSIS

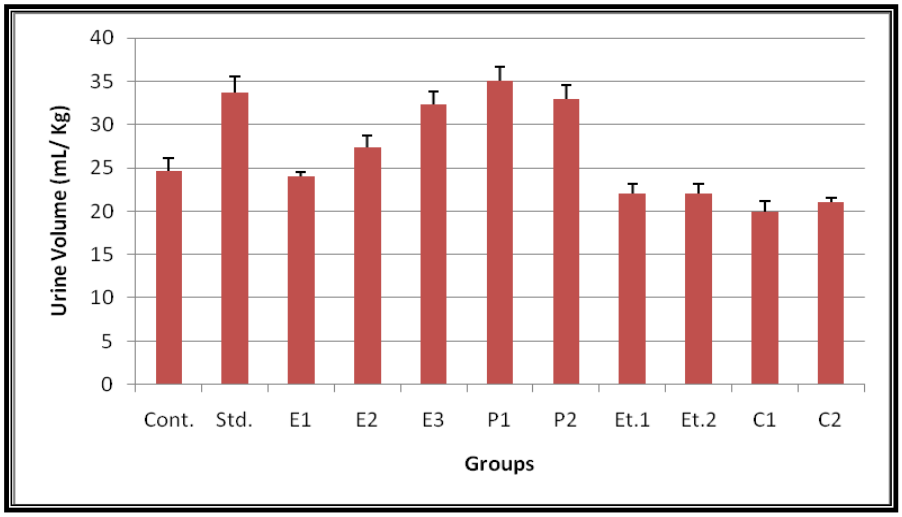
Statistical analysis by ANOVA followed by Dunnet's multiple comparison tests. Results are expressed as mean ± standard error, n = 3 in each group. Significant difference compared to control group at $p < 0.05$.

S.no.	Groups	Dose (mg/kg)	Urine vol. (ml/kg)	Conc. of excreted Na^+ ions (mEq/L)
1	Control (normal saline)(Cont.)	25 ml/kg	24.66 ± 1.452	78.63 ± 0.317
2	Standard (Furosemide)(Std.)	100 mg/kg	33.66 ± 1.855*	150 ± 1.154*
3	Ethanolic Extract (E_1) (E_2) (E_3)	300 mg/kg 400 mg/kg 500 mg/kg	24 ± 0.577 27.33 ± 1.452 32.33 ± 1.452*	81.66 ± 0.881 83.33 ± 0.881 85 ± 1.154*
4.	Pet. Ether Fraction(P_1) (P_2)	300 mg/kg 400 mg/kg	35 ± 1.732* 33 ± 1.527*	97 ± 1* 85.33 ± 2.027*
5	Ethylacet. Fraction ($Et._1$) ($Et._2$)	300 mg/kg 400 mg/kg	22 ± 1.154 22 ± 0.154	80.33 ± 0.881 80.33 ± 1.201
6	Chlorof. Fraction (C_1) (C_2)	300 mg/kg 400 mg/kg	20 ± 1.154 21 ± 0.577	85 ± 1.527* 81.66 ± 1.452

Graph no.4.1 showing concentration of excreted Na^+ ions (mEq/L)



Graph no. 4.2 showing amount of urine volume (ml/kg)



RESULTS

The results of the preliminary phytochemical screening of ethanolic extracts and its fractions are given in **Table No. 1**. The ethanolic extract was found to produce significant increase in excretion of sodium at the higher dose tested (500 mg/kg i. p.). The order of activity of increase in urinary output and urinary electrolyte excretion was found to be as petroleum ether fraction > chloroform fraction > ethyl acetate fraction.

DISCUSSION

The diuretic activity of ethanolic extracts as well as fractions (petroleum ether, ethyl acetate, chloroform) and Furosemide was carried out using Lipschitz test model. Statistical analysis was done using ANOVA followed by Dunnet's multiple comparison tests. Results were expressed as mean ± standard error, n = 3 in each group. Among all test compounds used, the petroleum ether fraction was found to be most active which shows 35 ± 1.732* ml/kg (Urine vol.); 97 ± 1* mEq/L (Conc. of excreted Na^+ ions) at 300 mg/kg dose and 33 ± 1.527* ml/kg (Urine vol.); 85.33 ± 2.027* mEq/L (Conc. of excreted Na^+ ions) at 400 mg/kg dose which is significant from the control group, at 0.05 % Probability. Results from the present study suggest that extract of *A. marmelos* possess diuretic activity. These results need further validation by doing experimental study in large scale. If the further studies support present study the effect can be further confirmed by clinical study. This plant may get place in near future in treatment of hypertension, renal failure, congestive heart failure and clinical conditions leading to edematous state of body.

REFERENCES

1. Holohan, P. D. General Principals and Renal Cellular Mechanisms of Drug Transport. In *Principles of Pharmacology, Basic Concepts and Clinical Applications*, 11th ed.; Manson, P. L., Ed.; Chapman and Hall, an International Thomson Publishing Company, New York, 1995; p 655.
2. Patel, P.; Asdaq, S. M. B. Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals. *Saudi Pharmaceutical Journal.* 2010, *18*, 161–165.
3. Nadkarni, A. K., Dr. K. M. Nadkarni's Indian Materia Medica, vol. 1. Popular Prakashan Company, Bombay (India), 1986, pp. 45–50.
4. Arul, V., Miyazaki, S., Dhananjayan, R. Studies on the antiinflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos. J. Ethnopharmacol*. 2005, 96, 159–163.
5. Arumugam, S., Kavimani, S., Kadalmani, B., Ahmed, A. B. A., Akbarsha, M. A., Rao, M. V. Antidiabetic activity

of leaf and callus extracts of *Aegle marmelos* in rabbit. *Sci. Asia*. 2008, 34, 317–321.

6. Shoba, F.G., Thomas, M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhea. *J. Ethnopharmacol*. 2001, 76, 73–76.
7. Vijaya, C., Ramanathan, M., Suresh, B. Lipid lowering activity ethanolic extract of leaves *Aegle marmelos* in hyperlipidaemic models Wistar albino rats. *Ind. J. Exp. Biol*. 2001, 47, 182–185.
8. Rana, B. K., Singh, U. P., Taneja, V. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves *Aegle marmelos. J. Ethnopharmacol*. 1997, 57, 29–34.
9. Ulahannan, R. K., Thomas, T., Sadasivan, C. Antibacterial action of leaves of *Aegle marmelos. Int. J. (Sci.)*. 2008, 2, 134–138.
10. Gangadevi, V., Muthumary, J. Taxol, an anticancer drug produced by an endophytic fungus Bartalinia robillardoides Tassi, isolated from a medicinal plant, *Aegle marmelos* Correa ex Roxb. *World J. Microbiol. Biotechnol*. 2008, 24, 717–724.
11. Kumar, R.; Kumar, A.; Prasa, C. S.; Dubey, N. K.; Samant, R. Insecticidal activity *aegle marmelos* (l.) correa essential oil against four stored grain insect pests. Int. *J. Food Safety*. 2008, *10*, 39-49.
12. Elango, G., Abdul Rahuman, A., Bagavan, A., Kamaraj, C., Abduz Zahir, A., Venkatesan, C. Laboratory study on larvicidal activity of indigenous plant extracts against Anopheles subpictus and Culex tritaeniorhynchus. *Parasitol. Res*. 2009, 104, 1381–1388.
13. Singh, S.; Rao, H, R. Hepatoprotective effect of the pulp/seed of *Aegle marmelos* correa ex Roxb. against carbon tetrachloride induced liver damage in rats. *Int. J. Green Pharmacy*. 2008, *2* (4), 232-234.
14. Kaur, P.; Walia, A.; Kumar, S.; Kaur. S. Antigenotoxic activity of polyphenolic rich extracts from *Aegle marmelos* (L.) correa in human blood lymphocytes and E.coli PQ 37. *Rec. Nat. Prod*. 2009, *3* (1), 68-75.
15. Vinodhini, R.; Narayanan, M. Cytoprotective effect of *Nelumbo nucifera* and *Aegle marmelos* in common crap exposed to heavy metals. *Int. J Integrative Biology*. 2009, 7(1), 124-129.
16. Patel, P.; Asdaq, S. M. B. Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals. *Saudi Pharmaceutical Journal.* 2010, *18*, 161–165
17. Sankari, M.; Chitra, V.; Silambujanaki, P.; Raju, D. Anticonvulsant activity of ethanolic extract of *Aegle marmelos* (leaves) in mice. *Int. J. Pharm. Tech. Res*. 2010, *2* (1), 640-643.
18. Devi, K.; Sivaraj, A. P.; Kumar, V.; Ahmed, K. S. Z.; Sathiyaraj, K.; Kumar, M. B. S.; David, E. Hypolipidemic effect of *Aegle marmelos* leaf extract in streptozotocin (stz) induced diabetic male albino rats *Int. J. Pharm Tech Res*. 2010, *2* (1), 259-265.
19. Sathiyaraj, K.; Sivaraj, A.; Madhumitha, G.; Kumar, P. V.; Saral, A. M.; Devi, K.; Kumar, B. S. Antifertility effect of aqueous leaf extract of *Aegle marmelos* on male *albino* rats. *Int. J. Current Pharm. Res.* 2010, *2* (1), 26-29.
20. Vimal, V., Devaki, T. Linear furanocoumarin protects rat myocardium against lipid peroxidation and membrane damage during experimental myocardial injury. *Biomed. Pharmacother*. 2004, 58, 393–400.

***Corresponding Author:**
Seema Singh
Department of Pharmacy,
Northern India Engineering College, Lucknow, (U.P.)