



LAXATIVE AND DIURETIC PROPERTY OF ETHANOLIC EXTRACT OF LEAVES OF *ALOCASIA MACRORRHIZA* LINN. ON EXPERIMENTAL ALBINO RATS

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ABSTRACT

The present study was carried out to evaluate the laxative and diuretic effect of *Alocasia macrorrhiza* leaves extract in rats. The ethanolic extract was found to produce significantly laxative activity in dose dependent manner. On other hand diuretic and natriuretic activities were carried out by administration of normal saline along with the treatment modules. The volume of urine (in ml) and the Na⁺ and K⁺ content in the urine were measured. The ethanolic extract of 100, 200 and 400 mg / kg, produced significant laxative, diuretic and natriuretic activity. Presence of different phytoconstituent in ethanolic extract of *Alocasia macrorrhiza* may be responsible for the specific activities. Overall, the extract was found to be significant laxative and diuretic activity.

KEY WORDS: *Alocasia macrorrhiza*, acute toxicity study, Diuretic activity, Laxative activity.

INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a variety of disease. There have little knowledge about their pharmacological action¹. Medicinal plants are important source of unknown chemical substance with potential therapeutic effect². Constipation also known as costiveness refers to bowel movement that is infrequent and/or hard to pass³. Constipation causes are two Types: obstructed defecation and colonic slow transit (hypo mobility)⁴. Constipation is a highly prevalent, after chronic gastrointestinal disorder that affects adult^{5, 6}. Laxative are among the most widely prescribed drug for the treatment of constipation⁷. Diuretics are the drug that increases urine volume; clinically useful diuretics also increase the rate of excretion of Na⁺ (natriuresis), Cl⁻ and water⁸. Diuretics are the first line drug for the treatment of hypertension. Diuretic has also major impact on the understanding of renal physiology⁹. *Alocasia macrorrhiza* (L)schott (family-Araceae, syn. *Alocasia indica*(L.) schott, *Alocasia macrorrhizos*) commonly known as manakachu and sholakachu in Bangladesh¹⁰. *Alocasia macrorrhiza* are naturally grown in marshy land of tropical area in India, Bangladesh, China and south Africa¹¹. The ethanolic extract of the leaves of the plant are used as antioxidant, anti-inflammatory, antinociceptive¹², antimicrobial¹³, antidiarrheal¹⁴, free radical scavenging activity¹⁵ and antiprotozoal. The hydroalcoholic extract of the leaves are used as hepatoprotective¹⁶ and anthelmintic activity¹⁷. The juice of the leaves given in colic, constipation, digestive, laxative, diuretic, astringent and rheumatic arthritis patient traditionally¹⁸. *Alocasia macrorrhiza* has antifungal¹⁹ and antitumour properties²⁰. Petioles contain HCN (up to 0.018%) used for toothache and their juice used for coughs²¹. Rhizomes are used for abdominal pain, vomiting and reduce elevated blood glucose level traditionally²².

The plant contains Oxalic acid, flavonoids, cholesterol, amino acids, gallic acid, mallic acid, ascorbic acid, succinic acid, glucose cyanogenetic glycosides, alocasin, fructose, sucrose, betalectins¹³, triglocholin, ceramide²³, isotriglocholin, β-glucosidases, phytosterol like compound²⁴, ergosterol, campesterol, stigmasterol, β-sitosterol and clionasterol²⁵. The spadix contain following

amino acid: leucine, α-alanine, proline, glutamic acid, glycine, valine, aspartic acid, γ-amino butyric acid, threonine and serine. Small amount of lysine, cystine, arginine, histidine, phenylalanine, tyrosine, glutamine and asparagines are also present. The present study aims at exploring the detail of laxative and diuretic action of ethanolic extract of *Alocasia macrorrhiza* leaves.

MATERIAL AND METHOD

Collection of plants

Fresh leaves of *Alocasia macrorrhiza* were collected from different places of Mahoba(Uttar Pradesh). Leaves of *Alocasia macrorrhiza* were authenticated and specimen was deposited at birbal sahni institute of paleobotany, lucknow

Preparation of extract

The leaves were dried in shade at room temperature and crushed coarse powder. The powder was passed through sieve number 14 to obtain a uniform sized powder; the powder was loaded in soxhlet apparatus and extraction process complete 30 cycles with ethyl alcohol (95%). After extraction the solvent distilled off by using vacuumed distillation. Extract was concentrated on water bath to dry residue²⁶.

Experiment animal

Wistar albino rats of either sex, weighing 170-200g provided by CDRI lab of lucknow, India. Before initiation of experiment, rats were acclimatized for a period of 7 day. Standard environment condition was maintained. The animal was allowed to standard pellet diet and tap water ad libitum. The experiment protocol has been approved by the institutional animal ethics committee and by regulatory body of the government.

Acute toxicity study

The acute toxicity of ethanolic leaf extracts of plant *Alocasia macrorrhiza* was determined using Swiss albino mice as per OECD 425 guideline, the animals were observed continuously for the behavioral changes for the first 2, 4 h and then observed for mortality if any, after 24 h.

Evaluation of laxative activity

Laxative activity was performed according to capasso et.al. On wistar albino rats of either sex, fasted for 12 hours before the experiment, but with water provided ad libitum. Rats were divided in five groups, each group consisting six rats.

The first group of animal, received normal saline (25ml/kg, p.o.), second group of animals received Agar-agar (300mg/kg, p.o.) the third, fourth and fifth groups of animals received simultaneously (100,200,400 mg/kg, p.o.) ethanolic extract of *Alocasia macrorrhiza*. After administration the animal were placed in a plastic container suitable for collection of faces. After 8 hours of drug administration, the faces were collected and weight. Thereafter, food and water were given to all rats and faecal outputs were again weight after a period of 16 hours⁴.

Evaluation of diuretic activity

Lipschitz test described by Lipschitz *et.al.* (1943) was employed for assessment of diuretic activity. In this method, wistar albino rats of either sex weight 150 to 200 gm were used. The rats were divided five groups of six animals each. The animals were fasted for 24 hours prior to the experiment and water was given ad libitum during fasting. The first group of animal, received normal saline (25ml/kg, p.o.), second group of animals received furosemide (20mg/kg, p.o.), third, fourth and fifth groups of animals received simultaneously (100,200,400 mg/kg, p.o.) ethanolic extract of *Alocasia macrorrhiza*. After administration the animal were placed in a metabolic cage (2 per cage), specially designed to separate urine and faces, and kept at 20°C±0.5°C. The volume of urine collected was measured at the end of 5 hours. During this period, no food and water was made available to animals. The parameter was taken volume of urine, electrolytes (Na⁺, k⁺, Cl⁻) were estimated in urine for assessment of diuretic activity. The Na⁺, k⁺, estimated was carried out using flame photometry. The Cl⁻ ion concentration was estimated by titration with 0.02N AgNO₃ using 5% potassium chromate solution as indicator²⁷.

Statistical analysis

All results are expressed as mean ± standard error. The data was analyzed using one ways of analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnet's test.

RESULT

The preliminary photochemical test revealed the presence of flavonoid, cholesterol, amino acids, glycoside and alkaloid in the ethanolic extract of *Alocasia macrorrhiza*. In laxative study, the different doses of the extract showed dose dependant increase in fecal output of rats when compared to the control group. However the test extract at lower dose (100mg/kg) failed to show the effect of laxative. The effect of *Alocasia macrorrhiza* at dose of 200 and 400 mg/kg, p.o. increased significantly fecal output of rats compared to control group. The laxative activity demonstrated by the test extract of 400mg/kg was significantly lesser than standard drug Agar-agar (300mg/kg,p.o.). The result is complied in the table 2. The present study revealed that, ethanolic extract of *Alocasia macrorrhiza* significantly increases the urinary output as well as urinary ion concentration at higher doses. However the test extract at lower dose (100mg/kg P.O.,) failed to do so. The ethanolic extract was found to produce significant increase in excretion of Na⁺, k⁺, Cl⁻ ions at the higher dose tested (400mg/kg p.o.). The order of activity of increase of urinary output was 400mg>200mg>100mg. The diuretic activity demonstrated by the test extract of 400mg/kg was significantly lesser than standard drug furosemide (20mg/kg). The result is complied in the table 1.

DISCUSSION

In the present study, we demonstrated the laxative effect of *Alocasia macrorrhiza* in a rat model of low-fiber diet-induced constipation. Maintaining a low fiber diet for 5

weeks significantly decreased stool frequency, weight and water content. A single administration of *Alocasia macrorrhiza* at 400mg/kg also significantly accelerated stool frequency, weight, and water content. Multiple administrations of *Alocasia macrorrhiza* at 200 and 400 mg/kg also significantly increased the frequency and weight of stools, and at 100-400 mg/kg, multiple administrations significantly increased stool water content. A single treatment of *Alocasia macrorrhiza* at 100 and 200 mg/kg did not show efficacy, but repeated treatment of *Alocasia macrorrhiza* at 200 mg/kg showed a significant increase in stool weight and stool frequency. These results indicate that *Alocasia macrorrhiza* ameliorated low-fiber diet-induced constipation in rats; therefore, *Alocasia macrorrhiza* may be suitable for human patients suffering from constipation due to their diet style. Diuretics are one of the groups of drugs used for the treatment of hypertension. Diuretics relieve pulmonary congestion and peripheral edema. They decrease plasma volume and subsequently venous return to heart. This decreases cardiac work load, oxygen demand and plasma volume, thus lowering blood pressure. They are the first line of drugs in the treatment of mild to moderate hypertension along with sodium restriction in the diet. The current study evaluated the diuretic potential of *Alocasia macrorrhiza* leaves in Wistar albino rats. The purpose of the present study was to establish the scientific basis for the traditional and the reported folk use of *Alocasia macrorrhiza* for diuresis. Ethanolic extract of *Alocasia macrorrhiza* leaves significantly increased the urinary output as well as urinary electrolyte concentration at a dose of 400mg/kg, p.o. but the effect was found to be less potent in increasing the urinary output when compared with the reference drug. Further the ethanolic extract of *Alocasia macrorrhiza* leaves were found to be more effective in enhancing urinary electrolyte concentration for all three ions tested (Na⁺, K⁺, Cl⁻).

The increase in the ratio of concentration of excreted Na⁺ and K⁺ ion indicated that the extract increasing Na⁺ ion excretion to a greater extent than K⁺ ion, which is very essential requirement of an ideal diuretic with lesser hyperkalaemic side effect.

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REFERENCES

- Balamurugan G, Selavarajan S, Balkrishnan D, Muralidharam P. Diuretic activity of *abutilon indicum linn* (sweet seed extract). J of herbal medicine and toxicology 2010; 4:49-52.
- Patel U, kulkarni M, Undale V, Bhosale A. Evaluation of diuretic activity of aqueous and methanol extracts of *lepidium sativum* garden cress (cruciferae) in rats. Tropical journal of pharmaceutical research 2009; 8:215-219.
- Chatoor D, Emmnauel A. Constipation and evacuation disorders. Best Pract Res Clin Gastroenterol 2009; 23: 517-30.
- Meite S, Bahi C, Yeo dodene, Date JY, Djaman JA, Guessan DJN. Laxative activities of *mareya micrantha*(Benth.)mull.arg. (euphorbiaceae) leaf aqueous extract in rats. Meite et al.BMC complementary and alternative medicine2010; 10:7.
- Muller-Lissner S. The pathophysiology Diagnosis and treatment of constipation. Dtsch Arztebl Int 2009; 106:424-432.
- Higgins PD, Johanson JF. Epidemiology of constipation in North America. A systematic review. Am J Gastroenterol 2004; 99:750-759.
- Bosshard W, Dreher R, SChnegg JF, Bula CJ. The treatment of chronic constipation in elderly people. An update Drugs Aging 2004; 21:911-930.
- Koti BC, Purnima A. Diuretic activity of extracts of *Centratherum anthelminticum*. international journal of green pharmacy 2008;2:228-231.

9. Tripathi KD. Essentials of medical pharmacology. Sixth edition. New Delhi: jay pee brother publishers; 2008.
10. Rahman MA, Solaiman M, Haque ME, Das AK. Analgesic and anti-inflammatory activities of *Alocasia indica*. Oriental Pharma Exp Med 2011; 11:1443-146.
11. Mandal P, Misra TK, Singh ID. Antioxidant activity in the extracts of two edible aroids. Indian j pharm sci.2010; 72:105-108.
12. Mulla WA, Chopade AR, Kuchekar BS. Antioxidant, antinociceptive, anti-inflammatory activities of ethanolic extract of leaves of *Alocasia indica*. J Young Pharm 2010; 2: 137-43.
13. Mulla WA, Sargade PB, Pawar AM, Tarkasband HA, Sayyad FJ. Antimicrobial activity of *Alocasia indica*. Int J Pharm Tech Res 2010; 2 : 327-33.
14. Mulla WA, Chopade AR, Bhise SB, Burade KB, Khanwelkar CC. Evaluation of anti-diarrheal and *in vitro* antiprotozoal activities of extracts of leaves of *Alocasia indica*. pharm bio 2011; 49: 354-61.
15. Mulla WA, Salunkhe VR, Kuchekar SB, Qureshi MN. Free radical scavenging activity of leaves of *Alocasia indica*. Ind J pharmaceu sci. 2009; 71: 303-07.
16. Mulla WA, Salunkhe VR, Bhise SB. Hepatoprotective activity of hydroalcoholic extract of leaves of *Alocasia indica*. Ind J Exp Biol 2009; 47: 816-21.
17. Mulla WA, Thorat VS, Patil RV, Burade KB. Anthelmintic activity of leaves of *Alocasia indica* linn. Int. J of pharmtech. Research 2010; 2:26-30.
18. Nadkarni AK, Nadkarni KM. Indian materia medica. Bombay: Popular prakashan private limited 1999.
19. Wang HX, Ng TB. Alocasin, An anti-fungal protein from rhizomes of the giant taro *Alocasia macrorrhiza*. Protein Expression and Purification 2003; 28: 9-14.
20. Ke Y, Zhou X, Bai Q. Antitumour effect of *Alocasia macrorrhiza*. Zhong yao cai 1999; 22: 252-3.
21. Asolkar LV, Kakkar KK, Chakre OJ. Glossary of Indian medicinal plants with active principles. 2nd edition. New Delhi: National Institute of Science Communication and information resources CSIR 1992.
22. Kirtikar KR, Basu BD. Indian medicinal plant. 2nd edition. Dehradun: New cannaught place 1975.
23. Tien NQ, Ngos P, Minh PH, Minh CH, Kimyh. New ceramide from *Alocasia macrorrhiza*. Arch pharma res. 2004; 27:1020-2.
24. Chopra RN, Chopra IC, Verma BS. Glossary of Indian medicinal plants. 1st edition. New Delhi: National Institute of Science Communication and information resources CSIR 1998.
25. Dinda B, Mohanta BC, Ghosh P, Sato N, Harigaya Y. Chemical constituent of *parkia javanica*, *Alocasia indica*, *prema latifolia*. J of ind. Che. Society 2010; 87:829-31.
26. Kumarasamyraja D, Shankar M, Gowrishankar NL. Preliminary phytochemical and diuretic Potential of methanolic extract of *azima tetra cahntham lam.*, leaf. International journal of pharmacy and industrial research 2011; 1:275-278.
27. Kane SR, Apte VA, Todkar SS, Mohite SK. diuretic and laxative activity of ethanolic extract and its fraction of *euphorbia thymifolia* linn. International Journal of ChemTech Research 2009; 1:149-152.

Table 1 : Diuretic Effect of ethanolic extracts of *Alocasia macrorrhiza* in rats.

Treatment	Urine volume (ml/100g/5h)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Na ⁺ /K ⁺
control	4.62±0.60	104.80±1.30	92.20±0.82	58.32±5.28	1.137
standard	8.26±0.46***	125.92±0.16**	62.01±2.11***	78.25±1.70*	2.031
100mg/kg ethanolic extract(n=6)	5.73±0.38 ^{ns}	112.58±3.50 ^{ns}	89.30±2.90 ^{ns}	55.30±3.25	1.261
200mg/kg ethanolic extract(n=6)	6.90±0.32*	118.20±3.12**	78.82±1.30***	60.83±5.37	1.499
400mg/kg ethanolic extract.(n=6)	7.68±0.60***	123.80±2.65***	72.40±1.30***	62.48±5.65	1.709

Values are given as mean±S.E.M;n=6, *P < 0.05, **P < 0.01, ***P < 0.001 considered for significance,(ANOVA followed by Dunnett's test) .

Table 2: Laxative activity of ethanolic extract of *Alocasia macrorrhiza* in rats

Treatment	Fecal output	
	8 hours	8-16hours
control	0.751±0.42	1.609±0.65
Agar-agar	5.086±1.12**	5.418±0.60**
100mg/kg ethanolic extract(n=6)	0.890±0.16	1.192±0.29
200mg/kg ethanolic extract(n=6)	3.705±0.78*	4.734±0.11
400mg/kg ethanolic extract(n=6)	4.825±0.91**	5.217±0.58**

Value are expressed as mean±S.E.M ;n=6, *P < 0.05 compared to control group;and; **P < 0.01 compared to control group.

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