

DIURETIC ACTIVITY OF *Centella asiatica* (L.) Urban

Muhammad Akhlaq^{1*}, Muhammad Khaleeq Alum², Syed Zahoor ul Hassan¹, Syed Rizwan¹, Muhammad Amjad chishti¹, Muhammad Mehboob Alam³

ABSTRACT: Diuretics are urinary output increasing water pills used as a co-therapy to treat edema, hypertension, lungs, liver and kidney dysfunctions and diabetes. *Centella asiatica* (L.) Urban is (Apiaceae) reported to possess all the above mentioned conditions. Therefore, it was evaluated for its diuretic activity by using metabolic cages for measuring urinary output after drug administration. The methanol extract of leaves and petioles of *C. asiatica* (L.) Urban plant was administered to Swiss albino mice, weighing between 22-28 g, in doses of 300 and 500 mg/ kg body weight. Acute oral toxicity test was also conducted in animals to find out the safety of drug at high doses. The test compound was found to be safe up to 4g/kg body weight therefore it can be used freely as herbal medicine. At both doses mild significant diuretic activity was observed and occurrence was dose and time dependent.

KEYWORDS: *Centella asiatica* (L.) Urban, Diuretic, Toxicity, Mice.

INTRODUCTION

Diuretics is a class of medicine also known as water pills given to increase urinary output as a result decreasing hypertensive and edemic conditions. It is specially prescribed in hypertension, congestive heart failure, liver cirrhosis, kidney dysfunction and diabetes. Commonly there are three types of diuretics: First type is thiazides including chlorothiazide, HCl-thiazide, metolazone and indapamide, second type is loop diuretics including torsemide, furosemide, ethacrynic acid and bumetanide while third type is potassium-sparing diuretics including amiloride, spironolactone, triamterene and eplerenone. Side effects of all these types are disturbed potassium and sodium levels in blood, headache, dizziness, thirst, diarrhea, allergic reaction and sometimes kidney failure (1).

This valuable medicinal herb is a part of traditional memory enhancing, venous hypertension, antioxidant, anti-inflammatory, antiulcer and wound healing medicinal products (Sushma et al., 2011). It contains important phytoconstituents such as Asiatic acid, asiaticoside, cadiyenol, castelliferol, catechin, centellasaponins, centelloside, corosolic acid, madecassic acid,

madecassoside, quercetin, rutin and ursolic acid. Keeping in view its use as an anti-inflammatory (2) venous hypertension (3), diabetes (4), cardiac (5), liver (6), lungs (7) and kidney diseases (8) traditionally also proven by certain scientific researches, it was decided to search *C. asiatica* methanol extract for its diuretic activity.

MATERIAL AND METHODS

C. asiatica (L.) Urban plant was purchased from local market of Karachi. The leaves with petiole were separated and methanol extract was prepared using reported method (9). Toxicity and diuretic activity on healthy, adult Swiss albino mice of both sexes weighing between 22-28 g procured from Animal House of Dow University of Health Sciences.

Toxicity Studies

Acute oral toxicity test was conducted on Swiss albino mice, weight limits 22-28g, according to the standard method (10). The animals were divided into three groups of six animals each (3 male and 3 female). All animals were kept in quarantine a week prior to study. The animals were fed with standard rodent diet and water at labitum. The animals of group 1 were given orally normal saline (0.5 ml), group 2, 3 and 4 received a single dose of *C. asiatica* plant extract in normal

1. Faculty of eastern medicine, Hamdard University Karachi.
2. Government Emerson college university Multan
3. School of Nursing, Pakistan institute of medical sciences (PIMS), Islamabad.

Corresponding Author:

muhammadakhlaq377@gmail.com

saline as 1, 2 and 4 g/kg body weight, respectively. The animals were observed for unusual movements immediately and after 30 minutes time interval for six hours after dose administration.

Diuretic Studies

The animals were divided into four groups comprising of three animals. Group 1 and Group 2 were test groups provided with 300 mg/kg and 500 mg/kg body weight dose of *C. asiatica* extract, Group 3 and Group 4 served as negative and positive control groups provided with normal saline (0.5 ml) and furosemide (10 mg/kg), respectively. The animals were kept separately after grouping in quarantine for 15 days with normal rodent diet and excess of water. Group 1 was given normal saline (0.5 ml) orally by means of graduated feeding cannula per animal and served as negative control group for comparison of results. The plant extract was mixed with normal saline and given to animals in doses of 300 and 500 mg/kg body weight. These two groups were marked as Group 2 and 3, while animals of Group 4 were given furosemide (50 mg/kg body weight) and served as positive control group. The animals after the administration of samples were kept in specially designed metabolic cages supplied by Techniplast, Italy Model Nalgene 170015. The water was present in excess and volume of urine passed was recorded after 2, 4 and 6 hours for each animal individually. The reading of test groups was compared with negative and positive control groups to find the study outcome.

RESULTS AND DISCUSSION

Toxicity Studies

All animals were active and alert showing normal symptoms while group 3 animals showed hyperactivity for some time and later normalized. No mortality was recorded. It

means the test sample is safe upto a dose of 4 g/kg body weight, therefore, can be used as a drug.

Diuretic Studies

Diuretic assessment was carried out in albino mice according to the procedure described in experimental section. The volume of urine output of individual animal in a group was measured and collective readings were recorded as mean±standard deviation for each group. The readings of both test groups were compared to the control group for analysis.

Table 1 shows the volume of urine in mice after drug administration. The test drug did not show any prominent diuresis at first two hours of administration as compared to control, while standard drug furosemide exhibited significant diuretic activity. At 4th and 6th hour the test drug showed marked increase in urinary output in dose dependent manner. The final volume of urine for normal saline receiving animals was 1.9 ± 0.07 , for 300 and 500 mg/kg dose of *C. asiatica* was 2.8 ± 0.32 and 4.2 ± 1.03 , respectively, and for standard marketed diuretic medicine furosemide it was 5.27 ± 1.86 .

Table 2 shows the difference in urinary volume obtained by subtracting the previous reading from current reading. The best result at 2nd and 4th hour was obtained by Furosemide while at 6th hour by *C. asiatica* extract at 500 mg dose.

CONCLUSION

C. asiatica methanol extract of leaves and petioles was safe up to 4 g/kg body weight therefore it can be used freely as herbal medicine. It possesses mild significant diuretic activity in dose and time dependent manner.

REFERENCES:

1. Qureshi M, Ahmad M, Mahjabeen NJ, Baig IA, Fatima N, Sharif H, et al. Diuretic Activity of *Centella asiatica* (L.) Urban. *MADINAT AL-HIKMAH*. 2017;60(3):15.
2. Huang S-S, Chiu C-S, Chen H-J, Hou W-C, Sheu M-J, Lin Y-C, et al. Antinociceptive activities and the mechanisms of anti-inflammation of asiatic acid in mice. *Evidence-Based Complement Altern Med*. 2011;2011.
3. Incandela L, Belcaro G, De Sanctis MT, Cesarone MR, Griffin M, Ippolito E, et al. Total triterpenic fraction of *Centella asiatica* in the treatment of venous hypertension: a clinical, prospective, randomized trial using a combined microcirculatory model. *Angiology*. 2001;52(2_suppl):S61–7.
4. Alqahtani A, Hamid K, Kam A, Wong KH, Abdelhak Z, Razmovski-Naumovski V, et al. The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and diabetic complications. *Curr Med Chem*. 2013;20(7):908–31.
5. Gnanapragasam A, Ebenezar KK, Sathish V, Govindaraju P, Devaki T. Protective effect of *Centella asiatica* on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats. *Life Sci*. 2004;76(5):585–97.
6. De Sanctis MT, Belcaro G, Incandela L, Cesarone MR, Griffin M, Ippolito E, et al. Treatment of edema and increased capillary filtration in venous hypertension with total triterpenic fraction of *Centella asiatica*: a clinical, prospective, placebo-controlled, randomized, dose-ranging trial. *Angiology*. 2001;52(2_suppl):S55–9.
7. Zhang L, Zheng J, Zhang L, Gong X, Huang H, Wang C, et al. Protective effects of asiaticoside on septic lung injury in mice. *Exp Toxicol Pathol*. 2011;63(6):519–25.
8. Pang LL, Hou LB, Mei QX, Kong XL, Hu Y, Gao YQ, et al. Effects of compound *Centella asiatica* enema on kidneys coefficient, electrolytes and blood in chronic renal failure rats. *Zhong yao cai= Zhongyaocai= J Chinese Med Mater*. 2010;33(5):775–8.
9. Fatima N, Ahmad M, Syed S, Imran H, Yaqeen Z. Antiulcerogenic and toxicological studies of *Glycyrrhiza glabra* roots available in local market of Karachi. *Karachi Univ J Sci*. 2008;36:5–8.
10. Schlede E, Mischke U, Diener W, Kayser D. The international validation study of the acute toxic class method (oral). *Arch Toxicol*. 1995;69:659–70.

Table 1: Diuretic Activity of *C. asiatica* at Different Time Points

Group	Sample	Dose (mg/kg)	0.5 hr	2 hr	4 hr	6 hr
1.	<i>C. asiatica</i> extract	300	0.22±0.03	0.62±0.21	1.9±0.02	2.8±0.32
2.	<i>C. asiatica</i> extract	500	0.31±0.02	0.5±0.11	2.2±0.04	4.2±1.03
3.	Normal saline	0.5 ml	0.32±0.04	0.8±0.03	1.0±0.05	1.9±0.07
4.	Furosemide	10	1.02±0.06	3.04±0.09	4.98±0.06	5.27±1.86

*Urinary output (ml)

Table 2: Diuretic Activity: Increase in Urinary Volume with Respect to Time in ml

Group	Sample	Dose (mg/kg)	0.5 hr	2 hr	4 hr	6 hr
1.	<i>C. asiatica</i> extract	300	0	0.40	1.28	0.90
2.	<i>C. asiatica</i> extract	500	0	0.19	1.7	2.00
3.	Normal saline	0.5 ml	0	0.48	0.20	0.90
4.	Furosemide	10	0	2.02	1.94	0.29