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Received on 22 October, 2013; received in revised form, 21 November, 2013; accepted, 13 February, 2014; published 01 March, 2014

DIURETIC ACTIVITY OF METHANOLIC RHIZOME EXTRACT OF *PICRORRHIZA KURROA* ROYLE EX. BENTH.

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Keywords:

Picrorrhiza kurroa, Diuretic activity, Furosemide, Kidneys

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ABSTRACT: Kidneys are the excretory organs of our body that serves the important functions such as elimination of waste products, regulation of fluid volume and electrolyte content of the extracellular fluid. *Picrorrhiza kurroa* (Scrophulariaceae) is a small perennial herb growing in the hilly parts of the north-Western Himalayas region in India and Nepal. The objective of the present study was to evaluate and compare diuretic activity of *Picrorrhiza kurroa* royle ex. Benth with that of the standard. Two doses of the test extract i.e. 300mg.kg and 500mg.kg body weight orally are used to evaluate the diuretic activity. Furosemide (20mg/kg i.p.) was used as the standard drug to compare the test results. The test extract showed significant diuretic activity ($p < 0.05$) which justify its use in ethnomedicine.

INTRODUCTION: Diuretics are the drugs that act on the kidney and are able to increase the volume of urine excreted, reason why are used in cardiac failure, chronic and moderate cardiac insufficiencies, acute oedema of the lung, nephritic oedema syndrome, arterial hypertension, diseases related with the retention of fluid etc.^{1, 2}. The diuretics act primarily by inhibiting tubular reabsorption and the drugs, which cause a net loss of Na⁺ and the water in urine are called diuretics. Furosemide is a sulphonamyl derivative which is a high efficacy diuretic which has its primary action on medullary ascending limb of loop of Henle and can produce substantial effect because of limited capacity for salt absorption in distal tubule and collecting duct.

Picrorrhiza kurroa Royle Ex. Benth belonging to the family Scrophulariaceae is a small perennial herb that is widely distributed in the north – West India on the slopes of Himalayas between 3000 and 5000mts^{3,4}.

Picrorrhiza kurroa is an important herb in the traditional Ayurvedic system of medicine and has been used to treat liver and bronchial problems. Other traditional uses include treatments of dyspepsia, bilious fever, chronic dysentery and scorpion sting. The most important active constituents of *Picrorrhiza kurroa* are the cucurbitacin glycosides, apocyanin, drosin, iridoid glycosides, picrosides and kutkin^{5,6}.

MATERIALS AND METHODS:

Plant collection, identification and authentication: The plant specimen was collected from S.V University, Tirupati, India and identified as *Picrorrhiza kurroa* Royle ex. Benth. Belonging to the family Scrophulariaceae, Voucher No: SDIP, Ref No: 002 dated 26/10/2012 and authenticated by

	<p>QUICK RESPONSE CODE</p>
	<p>DOI: 10.13040/IJPSR.0975-8232.5(3).961-63</p>
<p>Article can be accessed online on: www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(3).961-63</p>	

Dr. Madhavachetty, Botanist, Tirupati. The rhizomes of the plant were dried in vacuum oven at 40° C.

Preparation of plant extract: Rhizomes of *Picrorrhiza kurroa* plant is coarsely powdered and is successively extracted by continuous hot percolation method using Soxhlet apparatus employing methanol followed by distillation to recover the excess solvent. Methanolic extraction yielded sufficiently good quantity of the product. The extract was later subjected to drying and stored in a desiccator for further use⁷. The extract is soluble in water. Therefore, from the dried methanolic extract, accurately 300mg/ml and 500mg/ml solutions were prepared using distilled water.

Standard used for the activity: Furosemide was used as the standard drug to compare the test results. It was prepared in the concentration of 20 mg/kg in distilled water as the solvent.

Animals used for the study: Adult male Wistar rats (170-200 gms) were used for the study and kept at the laboratory animal house of Sree Dattha Institute of Pharmacy for acclimatization to laboratory environment. They were kept in well cross ventilated room at 27±2°C for 1 week before the commencement of experiment. Animals were provided with commercial rodent pellet diet and water ad libitum. Experiments were carried out as per the rules and regulations of CPCSEA.

Acute toxicity: Acute oral toxicity studies were performed as per OECD guidelines 425, dosed each animal at the dose of 3000mg/kg b.w.p.o. The animal was observed continuously for 2hrs for gross behavioral changes and intermittently once every 2hrs and finally at 24 and 72hrs to note any signs of toxicity including death.

Assessment of diuretic activity: Male Wistar rats (175 - 200 gms) were maintained under standard conditions of temperature and humidity. The method of Lipschitz *et al*⁸ was employed for the assessment of diuretic activity. Four groups of six rats each were fasted and deprived of water for 18hrs prior to the experiment. On the day of experiment, normal group of animals were given distilled water orally (25ml/kg body weight) and the treated groups were given 300mg/kg and 500mg/kg body weight of the methanolic extract of *Picrorrhiza kurroa*. The standard group was given furosemide (20mg/kg) intraperitoneally. The rats were placed in metabolic cages specially designed to separate faecal matter and urine. The urine volume was registered at 1, 2, 4, 6 and 24 hrs. post administration. During this period no food or water was given to the animals. The total urine volume was measured for both control and treated animals. The sodium, potassium and chloride ion concentration in the urine samples were determined and tabulated in **Table 1**.

TABLE: 1 EFFECT OF DIURETIC ACTION OF METHANOLIC EXTRACT OF PICRORRHIZA KURROA ROYLE EX. BENTH

Group	Dose (mg/kg)	Electrolyte concentration in m eq/l			
		Na ⁺	K ⁺	Cl ⁻	Na ⁺ /K ⁺
Control	25ml/kg	176.27 ± 12.78	87.24 ± 10.53	113.2 ± 8.9	2.02
Standard	20mg/kg	324.63 ± 12.45	123.56 ± 10.09	215.7 ± 11.4	2.62
MRPK	300mg/kg	220.60 ± 7.98	99.61 ± 9.8	123.87 ± 9.7	2.21
MRPK	500mg/kg	274.57 ± 8.34	105.45 ± 6.98	147.87 ± 8.7	2.60

Results are expressed as Mean ± SEM; n=5 in each group; *p<0.05, MRPK is methanolic extract of *Picrorrhiza kurroa*

RESULTS AND DISCUSSION: The % yield of methanolic extract of rhizomes of *Picrorrhiza kurroa* after 24hrs of hot percolation was found out to be 34%. The preliminary phytochemical screening showed the presence of carbohydrates, glycosides, saponins, steroid like phytochemical constituents. Cucurbitacins, Phenolic, Iridoid glycosides are some of the principle constituents responsible for various pharmacological activities. The presence of saponins and iridoid glycosides

like kutkin, Picroliv, Picrisides I, II, III & IV, Kutkosides are the chemical moieties that may be responsible for diuretic activity⁹. Many herbal diuretics exert their action by directly affecting the electrolyte balance of minerals. In the normal rats diuresis began with low volumes of urine excreted until completing 24hrs. The level of Na⁺ and K⁺ was equally low. The furosemide (positive control) treated group, the diuretic action started 60minutes and increased significantly from normal rats.

The increase in the ratio of concentration of sodium and potassium ions indicate that the extract increases sodium ion excretion to a greater extent than potassium which is an essential quality of good diuretic with lesser hyperkalemic side effect¹⁰. The chloride excretion was not elevated significantly by the lower dose and the results are indicating that the extract is a potent natriuretic¹¹. The test extract showed diuretic effect after the administration of 300mg/kg and 500mg/kg body weight dose. Out of both the doses 500mg/kg has shown noticeable diuretic property resulting in the superior urine excretions of Na⁺ and K⁺ ions which can be compared to the positive controls.

ACKNOWLEDGEMENTS: Authors express their sincere gratitude to the management, Directors of Sree Dattha Institutions for their timely support in providing us the necessary facilities for carrying out the research work.

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How to cite this article:

Rajani A, Reddy MVV, Madhuri M, Hemamalini K and Swathi M: Diuretic activity of methanolic rhizome extract of *Picrorrhiza kurroa* Royle Ex. Benth. *Int J Pharm Sci Res* 2014; 5(3): 961-63.doi: 10.13040/IJPSR.0975-8232.5(3).961-63

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