IJPSR (2010), Vol. 1, Issue 8 (Suppl.) **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 April, 2010; received in revised form 18 June, 2010; accepted 29 July, 2010

STUDIES ON THE ANTI- INFLAMMATORY PROPERTIES OF *CURCULIGO ORCHIOIDES* GAERTN. ROOT TUBERS

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Keywords:

Curculigo orchioides, Methanolic Extract, Anti-inflammatory Effect, Carrageenan induced rat paw oedema

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ABSTRACT

In this study, the anti-inflammatory effect of methanolic extract obtained from root tubers of plant *Curculigo orchioides* Gaertn. (Amaryllidaceae) was investigated. The effect of extract was studied in Carrageenan induced rat paw oedema. The extract at a dose of 200mg/kg and 400mg/kg was carried out for the investigation. The results indicate that methanolic extract, at the dose of 200mg/kg & 400mg/kg was found to have statically significant anti-inflammatory activity as compare to control. The percentage inhibition of inflammation was found higher at the dose of 400mg/kg body weight at 3rd hr as compare to 200mg/kg.The activity was compared with that of the standard drug, Diclofenac sodium (15mg/kg).

INTRODUCTION: Plant, as illustrated throughout the history of civilization has served as the major source of medication for the treatment of human ailments. Herbal medicines are being accepted and used increasingly by general populations in both eastern and western countries not only as medicines but also as dietary supplements, along with modern chemotherapeutic agents. This is because of ethnic acceptability and compatibility having fewer side effects. Chronic inflammatory diseases including rheumatoids arthritis are still one of the main health problems of the world's populations. Although several modern drugs are used to treat these types of disorders but their prolonged use may cause several adverse side effects [1]. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects [2]. Several plants are being used in traditional medicines for treating these disorders which are inflammatory in nature like rheumatism, arthritis, etc.

Curculigo orchioides is a small, annual herb found in the subtropical Himalayas from Kumaon eastwords and in the Western Ghats from Kinkan South words [3]. It belongs to family Amaryllidaceae commonly known as Kalimusli (In Hindi), in Orissa as Talamuli [4]. It consist of blackish elongated tuberous root having several lateral roots with rosette or short petiolate, linear, lanceolate, membraneous leaves close to the ground level. It was first introduced in 'Charak Samhita' of 'Agnivesha', the epic treatise of the medicine school of thought of the Hindu system of medicine and narrated as an ingredient of a cigar to alleviate cough. Talamuli has been used in the indigenous system of medicine for long periods. According to Bhavaprakash the drug is sweet, bitter, acts as an aphrodisiac. In Raj Nighantu it has been described as sweet, cooling, mucilaginous, incrases Kapha and reduces Pitta daha (burning sensation), acts as stimulant, gives strength. Musali prepared as a paste with goat's milk or honey and applied locally over the face, brightens the complexion of the face.Moving in to the modern period it is extensively used by the Ayurvedic practitioners, particularly ingredients of aphrodisiac preparations. The root tubers contain various types of saponins, the phenolic glycosides, resins, tannins, and polysaccharides [5-8]. The objective of the present investigation was planned to find out the therapeutic level of methanolic extract of *C. orchioides* Gaertn. root tubers in Anti-inflammatory activity (Carrageenian induced rat paw oedema).

MATERIAL AND METHODS: The powder root tubers of *Curculigo orchioides* collected from the rural belt of Barpali, Orissa in month of August-September and authenticated by Botanical Survey of India, Howrah, Kolkata (Reference no. CNH/I-I(5)/2009/ Tech. II/35). After authentication, fresh plant material was collected in bulk, washed under running tap water to remove adhering material, dried under shade and pulverized in a mechanical grinder. The coarse powder was passed through sieve no. 40 and taken for further studies.

Preparation of Drug Extract: The powder root tubers were extracted successively with pet. ether, chloroform, ethyl acetate & methanol in soxhlet extraction apparatus (Hot extraction). 300gm of dried coarse powder of *C. orchioides* root tubers were successively extracted by using solvents of increasing polarity i.e. Pet. Ether (60^0 – 80^0C), Chloroform, Ethyl acetate and Methanol. The extract was filtered while hot and the resultant filtrate was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a dessicator till experimentation. The successive methanolic extract (deep brown color) was filtered & dried under reduced pressure to get a solid mass free from the solvent. The yield was 5.9% with respect to dry starting material with characteristic odor &

greasy consistency. The dried extract was dissolved in solution of 2% gum acacia in distilled water (vehicle) for the evaluation of anti-inflammatory activity.

Animals: Adult Wistar albino rats weighing between 150-200gm of either sex. The animals were maintained under normal laboratory condition & kept in standard polypropylene cages at room temperature of $30^0 \pm 2^0$ and 60 to 65% relative humidity and provided with standard diet & water *ad libitum.* The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from in house ethical committee has been taken to carry out (Reference no. PCB/AEC/04/09) and complete this study.

Screening of Anti-inflammatory Activity (Carrageenan induced paw oedema): Anti-inflammatory activity was assessed using Carrageenan induced paw oedema method. The selected animals were divided into four groups of six rats each. The animals were starved overnight and were given the following treatment. Group I (Control group) received 1ml 2% w/v gum acacia solution orally, group II received 15mg/kg of Diclofenac sodium orally and group III & group IV received methanolic extract of *C. orchioides* at a dose of 200mg/kg and 400mg/kg respectively. After one hour, the rats were challenged by a subcutaneous injection of 1ml of 1% w/v solution of carrageenan into the subplantar side of the left hind paw. Paw edema was measured by wrapping a piece of cotton thread round the paw and measuring the circumference with a Meter rule [9, 10]**.** Measurement was carried out immediately before and different time intervals (1^{st} hr, 2^{nd} hr, 3^{rd} hr, 4^{th} hr & 5^{th} hr) following carrageenan injection. The difference between the initial and subsequent reading gave the acute oedema volume (**table 2**). The percentage of inhibitory activity at 5^{th} hr was calculated according to the following formula [11].

$$\text{Percent Inhibition} = 1 - V_t / V_C \times 100$$

Where, 'Vt' represents oedema volume in test materials and 'Vc' represent oedema volume in control.

Statistical Analysis: The mean value ± SEM was calculated for each parameter. The results were analyzed statistically by ANOVA is followed by Dunnet's test. The significant level was fixed at $p < 0.01$ & $p < 0.05$. The results of experiments by proper statistical analysis as stated above are tabulated in **table 1**.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF *CURCULIGO ORCHIOIDES* GAERTN. ROOT TUBERS IN CARRAGENNAN INDUCED PAW OEDEMA

Group	Treatment	Dose	Increase in Paw Volume in cm				
			1^{st} hr	2^{nd} hr	3^{rd} hr	4^{th} hr	5^{th} hr
I	Control (Carrageenan)	2ml/kg	2.189±0.020	2.311±0.044	2.511±0.022	2.456±0.026	2.256±0.044
II	Standard (Diclofenac sodium)	15mg/kg	2.133±0.024	2.200±0.034	2.167±0.029*	2.178±0.028*	2.156±0.014
III	Test Drug – I	200mg/kg	2.122±0.020	2.200±0.038**	2.256±0.040*	2.200±0.049*	2.133±0.024**
IV	Test Drug - II	400mg/kg	2.133±0.034	2.178±0.014	2.189±0.026*	2.200±0.057*	2.133±0.042**

Each value represents the mean ± S.E.M., n=6, *P<0.01, **P<0.05 compared with control, Dunnett's test after analysis of variance

TABLE 2: PERCENTAGE OF INHIBITION IN CARRAGENNAN INDUCED RAT PAW OEDEMA

Treatment	Percent Inhibition				
	1st hr	2nd hr	3rd hr	4th hr	5th hr
Standard (Diclofenac sodium)	29.63	35.69	67.32	16.96	39.00
Test Drug – I (200mg/kg)	35.45	35.69	49.90	56.14	48.05
Test Drug - II (400mg/kg)	29.63	42.77	63.01	56.14	48.05

RESULT: The result obtained as mean increase in paw volume (cm) and percentage inhibition is represented in **table 1 & 2**. The results indicate that methanolic extract, at the dose 200mg/kg p.o. & 400mg/kg was found to have statically significant anti-inflammatory activity as compare to control. The percentage inhibition of inflammation was found higher at the dose of 400mg/kg body weight at 3rd hr as compare to 200mg/kg.

DISCUSSION: Anti-inflammatory activity of an *C. orchioides* root tubers methanolic extract can be determined by their ability to reduce or prevent oedema [12]. Oedema represents the early phase of inflammation in carrageenan induced paw edema and is the simplest and most widely used model for studying anti-inflammatory activity. The paw edema induced by the subplanter injection of carrageenan in rats is biphasic phenomenan [13]. The first phase of oedema is attributed to release of histamine and serotonin, while the second phase (over 1hr) is mediated by prostaglandins like substance, the cyclooxygenase products and the continuity between two phases is provided by kinnins [14]. The knowledge of these mediation involved in different phases is important for interpreting mode of drug action, with the knowledge of these mediators involved in different phases of inflammation, the observed significant anti-inflammatory activity of methanolic extract (200mg/kg & 400mg/kg) of *C. orchioides* root tubers at significant hr may possible due to inhibition of kinin and prostaglandin biosynthesis enzymes responsible for plateau and second phase accelerating phases of inflammation, increased vascular permeability or by any other mechanism.

Further, the anti-inflammatory activity is due to presence of phytoconstituents such as alkaloids, polyphenolic compounds (flavonoids), tannins and sterols, to name a few. The methanolic extract of *C. orchioides* root tubers shows good anti-inflammatory activity and it can be attributed to the presences of tannins. Tannins are reported to inhibit the prostaglandin sysnthesis [15, 16]. All these studies will be of immense use in carrying out further research and revalidation of its use in Ayurvedic system of medicine.

ACKNOWLEDGEMENT: The Authors are grateful to extend special thanks to Mr. R.L. Hota, Chairman, G.B of The Pharmaceutical College, Barpali for his constant encouragement & support throughout the work. The authors extends there sincere thanks to Mr. N. K. Hota, President & Mr. S.K.Sahu, Secretary of The Pharmaceutical College, Barpali for providing all kind of facilities for this work.

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