International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.1, pp 99-104, Jan-Mar 2013

Bronchorelaxent And Anti-Inflammatory Effect Of Heteropogon contortus (L.) Beauv. Methanolic Extract

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Abstract: Methanolic extract of *Heteropogon contortus* (L.) Beauv was screened for Bronchorelaxation (in vitro anti-histaminic) and *in vivo* anti-inflammatory effects. Bronchorelaxation effect was evaluated in terms of inhibition of histamine and acetylcholine induced contraction in guinea pig tracheal chain. Anti-inflammatory effect was assessed using carrageenan and egg albumin induced inflammation in rat paw edema model. HC-ME shown 24.02 percent inhibition of inflammation in carrageenan induced paw edema model at 200 mg/kg. At same dose extract inhibited 29.27 percent inflammation in egg albumin induced edema model. Percent bronchorelaxation exhibited by extract against histamine and acetylcholine induced contraction was found to be 40.63 and 45.81 respectively. While insignificant results were found in terms of bronchprotection effect of extract.
Key Words: *Heteropogon contortus,* Bronchorelaxation, Anti-inflammatory.

INTRODUCTION

Heteropogon contortus (L.) Beauv. ex Roem. and Schult. Synonym *Andropogon contortus* L. belonging to the Family Poaceae (alt. Gramineae), **HC**. The grass is commonly known as common spear grass, black spear grass, bellary grass, *Kher* (Hindi), *Gantegawata* (Marathi). Its native distribution encompasses southern Asia, southern Africa and Norhern Australia. The species has also become a naturalised weed in tropical and subtropical regions in Asia and the east Americans[1].

Grass is reported to contain myo-inositol, galactinol, and raffinose It is reported to contain polysaccharide[2, 3]. Roots have been reported to have diuretic and stimulant properties. Plants also reported to found useful in toothache, fever, atrophy, emaciation or cahexy, muscular pain, hematological disorders, dysentery and scorpion sting[4]. It is ethnopharmacologically used for asthma in the form of extracts or steam distillation product of whole plant[5]. Oil distilled from the awns has been found useful in asthma. Recently we have reported the mast cell, cell membrane and free radical stabilization potential of methanolic extract of grass. As a part of continuous pharmacological evaluation of medicinally important plants this papers is an attempt to evaluate the bronchorelaxent and anti-inflammatory effect of extract.

MATERIALS AND METHODS

Animals

Albino rats and guinea pigs were housed under standard 12:12 h light/dark cycle in a temperature controlled (24 ± 1^{O}C) environment with

ad libitum access to rodent chow (Lipton, India) and water. All experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) Constituted for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi (IAEC approval No. 536/02/C/CPCSEA).

Collection and Authentication of *Heteropogon contortus*

Heteropogon contortus (Linn.) P. Beauv. *ex* Roem. and Schult. grass, belongs to the family Poaceae; were collected from the campus of Rashtrasant Tukadoji Maharaj Nagpur University campus, Nagpur as well as outskirts of Nagpur District, India, and authenticated from Botanical Survey of India, Pune, India. [Reference No. BSI/WC/Identi./Tech./2008/473 and 272].

Extraction of HC Grass

Coarse powder (500 g) of HC was exhaustively defatted using pet ether ($60\text{-}80^0$) and then extracted with methanol using Soxhelet apparatus to obtain HC-PE and HC-ME respectively. Extracts were concentrated, filtered through Whatmann filter paper 41 and stored in air tight container placed in vacuum desicator for future use.

Evaluation of Anti-Inflammatory Effect

Carrageenan induced rat paw edema

Albino rats of either sex were fasted for 24 h before the commencement of experiment. The test extracts randomly selected in two different doses (i. e. 100 and 200 mg/kg) while standard anti-inflammatory drug indomethacin (10 mg/kg) were suspended in 1 % CMC and administered orally by intra gastric tube 1 h before the inflammation induction. Inflammation was induced in hind paw of rats by injecting freshly prepared carrageenan suspension (0.1 ml; 1 % w/v)[6]. Change in paw volume was recorded at 1, 3 and 5 h after the carrageenan injection and compared against control group to calculate percent inhibition by formula- [% Inhibition of inflammation = (paw edema volume control-sample/control) X100].

Egg albumin induced rat paw edema

Albino rats of either sex were fasted for 24 h before the commencement of experiment. The test extracts in two different doses (i. e. 100 and 200 mg/kg) while standard anti-inflammatory drug indomethacin (10 mg/kg) were suspended in 1 % CMC and administered orally by intra gastric tube 1 h before the inflammation induction. Inflammation was induced in hind paw of rats by injecting 0.1 ml/kg of fresh egg albumin into the plantar region of the hind paw[7]. Separate sets of rats (n = 5) were employed for control, standard and treatment groups as described in carrageenan induced inflammation experiment. The change in paw volume (in mm) was measured up to 120 min, at 20 min intervals after egg albumin injection. % inhibition of inflammation was calculated as described above. Treatment, standard and test group protocol was retained as per the previous study.

Evaluation of Bronchodilation effect

Guinea pigs of either sex were sacrificed by a blow on the head and exsanguinated. The section of trachea was separated from adjacent tissue to obtain tracheal rings which were tied to get chain of 3-4 individual tracheas. The chain was mounted in a 20 ml organ bath containing Krebs–Henseleit (K–H) solution of the composition (mM): NaCl, 118.4; KCl, 4.7; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; $CaCl_2$, 2.5, $MgSO_4$, 1.2; glucose, 11.1; pH 7.4 ± 0.05 and temperature of bath was maintained at 37±1ºC. Tracheal chain was suspended under isotonic tension of 0.5 g and allowed to equilibrate for at least 1 h before commencing the experiment. During the experiment K-H solution was replaced after every 10 min. After the equilibrium period contraction was induced by adding the acetylcholine or histamine. Thereafter, the test extracts (1 mg/ml) was added serially (0.1, 0.2 up to 0.6 ml) in increasing doses and observed for bronchodilation. At the end of experimentation, the effect of test extract on pre-contracted tracheal chain was expressed as percent inhibition of contraction (bronchodilation) [8].

Evaluation of Bronchoprotective Effect

Guinea pigs were fasted for 12-24 h and only water was provided *ad libitum* before the commencement of experiment. Animals were screened for the sensitivity and suitability for study by challenging with mixture of equal volume of 0.1% histamine hydrochloride and 2% acetylcholine chloride, under the average pressure of 450±50 mmHg for 15 sec in a plexiglass chamber (histamine chamber). The time to onset of respiratory distress (preconvulsive time in sec) during the aerosol challenge was measured. Guinea pigs were considered to be insensitive and discarded with preconvulsive time of more than 120 sec. The adequate and sensitive guinea pigs were randomly allotted to different groups (control, treatment and

standard, described below) with 04 per each. The negative control of animals administered 0.1 % CMC, 5 ml/kg, the positive control animals administered aminophylline (10 mg/kg) suspended in 0.1 CMC and test extract groups were administered with (100 and 200 mg/kg, suspended in 0.1% CMC). All animals were treated with a single dose of extracts and aminophylline, daily for 3 days prior to the challenge while the last dose was administered 1 h before the bronchial challenge. The delitescence of convulsion for each animal and tumble numbers for each group during challenge within a 6 min exposure period were recorded. Aerosol provoked a bronchospastic reaction in all animals within three minutes. The delay in the appearance of the bronchospastic reaction was considered as bronchoprotective effect and bronchoprotection from convulsion was expressed relative to control.[8]

Percentage protection= $[1-(T_1/T_2)]$ X 100. Where, T_1- preconvulsive breathing time (sec) in control group and T_2 - Preconvulsive breathing time (sec) treatment or standard group.

RESULTS AND DISCUSSION

Our previous research reports the ability of HC-ME extract to scavenge free radical, mast cell stabilization (*in vitro* anti-histamine activity) and membrane stabilization (*in vitro* anti-inflammatory activity). This paper also reports the chromatographic fingerprinting and quantitative spectroscopic standardization of extract[9]. The same extract was screened for the bronchorelaxent (*in vitro* anti-cholinergic and anti-histaminic) and anti-inflammatory effect.

Among the several contributing factors, two imperative targets that should be monitored in the management and cure of asthma are aggravated inflammatory conditions and bronchoconstriction[10]. Therefore, present study endeavored to evaluate *in vivo* anti-inflammatory and bronchoprotective potential of HC-ME extract.

In carrageenan induced inflammation study, HC-ME at the dose of 100 mg/kg inhibited only the initial phase of inflammation, while initial as well as lateral phases of inflammation were inhibited by HC-ME at the dose of 200 mg/kg with percent inhibition of inflammation 19.90, 24.02 and 19.07% respectively at 1, 3 and 5 h (Table 02).

The carrageenan-induced rat paw edema is categorized into two phase phenomenon based on the time, release and the type of mediators involved. The first hour after carrageenan injection is considered as an initial phase which is attributed to the release of histamine and 5-HT, while 3-5 h after carrageenan injection is the second phase and contributed by induction of prostaglandins, bradykinins, protease and lysosome, and mediates of edema formation[6].

Earlier studies have indicated the use of egg-albumin as a phlogistic agent and can be used to screen anti-inflammatory agents[7]. As, the phenomenon of inflammation induced by egg albumin is considered similar to the carrageenan, only difference and advantage of the egg albumin model is ability to facilitate the measurement of inflammation and inhibition of same between 20-120 min at 20 min interval.

To substantiate the anti-inflammatory activity, the efficacy of these three WF extracts was also studied against egg albumin induced inflammation. The HC-ME (100 mg/kg, p.o.) exhibited anti-inflammatory effect ($P>0.05$) at 20 and 80 min time interval (initial phase) but showed lack of significant inhibition of inflammation at 60, 100 and 120 min after egg albumin administration as compared to control. On the other hand, the HC-ME (200 mg/kg, p.o.) significantly inhibited ($P<0.05$) egg albumin induced inflammation at all time interval (initial as well as lateral phase) (Table 03).

Thus, it can be depicted that, HC-ME at higher dose (200 mg/kg) exhibited significant anti-inflammatory profile in both the models of inflammation; the plausible mechanisms for the observed anti-inflammatory activity of HC-ME might be attributed to inhibition of synthesis or release and action of major inflammatory mediators like histamine, serotonin, bradykinins and prostaglandins. The anti-inflammatory activity can be correlated with presence of important bioactive phytoconstituents of these extracts steroids and triterpenoids which has been reported to have anti-inflammatory activity. The anti-inflammatory effect of HC-ME was compared with the reference standard i.e. indomethacin, against both carrageenan and egg albumin inflammation. Observed anti-inflammatory effect might be contributed by the presence of triterpenoids and saponin in the extract as these phytoconstituents have been reported to exhibit anti-inflammatory effect[11].

HC extract exhibited bronchorelaxation against histamine and acetylcholine (both at 1 µg/ml) induced bronchoconstriction. HC-ME (1 mg/ml) relaxed precontracted tracheal preparations to varying degrees, when added cumulatively to organ bath containing acetylcholine and histamine precontracted tracheal chain. HC-ME at 0.6 ml exhibited maximum relaxation of 45.81% in acetylcholine while at similar dose observed relaxation was 40.63 observed in histamine induced contraction (Table 04).

Table 1: Percentage yield and weight of HC extracts

Plant material	**HC whole plant**	
Extracts obtained	**HC- ME**	**HC -PE**
Weight of extracts (g)	11.56	7.6
% Yield	2.89	0.19

Table 02: Anti-inflammatory activity of HC-ME and indomethacin in carrageenan and egg albumin induced inflammation model

Treatment	**Dose (mg/kg)**	**Measurement of paw volume and % inhibition of inflammation at (h)**		
		01	**03**	**05**
Control	**----**	6.23 ±0.13	7.45±0.14	6.50±0.19
HC-ME	**100**	5.97±0.25[4.17]	6.47±0.41 [13.15] *	5.38±0.89 [17.23]*
	200	4.99±0.22 19.90]*	5.66±0.67 [24.02]*	5.26±0.87 [19.07]*
Indomethacin	**10**	5.05±0.17[18.97]*	3.15±0.15[57.72] **	3.67±1.02[43.53] **

Values represent the mean ± S. D. of five animals for each group, value in square bracket indicates the percentage inhibition rate of inflammation, ns- non significant, P< 0.01* and P< 0.001 ** indicates different level of statistically significant values against control

Table 03: Anti-inflammatory activity of HC-ME and indomethacin in egg albumin induced inflammation model

Treatment	**Dose (mg/kg)**	**Measurement of paw volume and % inhibition of inflammation at (min)**					
		20	**40**	**60**	**80**	**100**	**120**
Control	**----**	5.45±0.12	5.96± 0.23	6.62± 0.17	7.67± 0.21	7.30± 0.33	7.14± 0.19
HC-ME	**100**	5.02±0.54 [7.88]	5.12±0.66 [14.09] *	6.28± 0.57 [5.13]	6.45± 0.82 [15.90] *	6.89± 0.73 [5.61]	6.78±0.77 [5.04]
	200	4.16±0.62 [23.66] *	4.96±0.51 [16.77] *	5.19±0.61 [21.60] *	5.59±0.86 [27.11]**	6.01±0.49 [17.67] *	5.05±1.01 [29.27]**
Indomethacin	**10**	4.79±0.13 [18.41] **	5.27± 0.12 [11.58] **	5.53±0.23 [16.43] **	5.76± 0.16 [24.96]**	3.93± 0.18 [46.15] **	3.25± 0.14 [54.48]**

Values represent the mean ± S. D. of five animals for each group, value in square bracket indicates the percentage inhibition rate of inflammation, ns- non significant, P< 0.01* and P< 0.001 ** indicates different level of statistically significant values against control

Table 04: Effect of HC extracts and aminophylline on acetylcholine and histamine precontracted guinea pig tracheal chain

Extract/ Drug added (ml)	**Acetylcholine induced contraction Vs treatment**		**Histamine induced contraction Vs treatment**	
	AMN	**HC-ME**	**AMN**	**HC-ME**
0.1	18.17±3.42	05.14± 1.91	18.82±2.31	NO EFFECT
0.2	41.98±2.75	11.78±2.56	44.76±3.78	03.09±1.92
0.3	68.25±3.56	23.33 ±2.71	78.25±3.56	10.31±2.52
0.4	97.83±3.09	32.50±2.11	100±3.85	21.171.85
0.5	97.83±3.09	41.08±2.87	100±3.85	32.85±3.11
0.6	97.83±3.09	45.81± 2.38	100±3.85	40.63±2.86

Values represent the mean ± S. D. of three replicates for each group.

Table 05: Bronchoprotective effect of HC-ME and aminophylline on acetylcholine and histamine aerosol induced bronchospasm in guinea pigs

Treatment groups	Dose (mg/kg)	Tumble No.	Latency (sec)		% Protection
			Before treatment	After treatment	
Control	---	14	88 ±13	89 ±14	
HC-ME	100	14	84 ± 17	85 ± 14	3.52
	200	12	80 ± 12	89 ± 10	10.93^{ns}
Aminophylline	10	08	90 ± 18	162± 27**	45.44

Values represent the mean ± S. D. of four independent replicates.
**p < 0.001 indicates the significance level when compared against control group.

Bronchoconstriction is associated with reversible obstruction of airways and considered as characteristic feature of bronchial asthma. Acetylcholine released from efferent nerve ending of inner bronchus results in the excessive formation of inositol 1, 4, 5- triphosphate (IP_3) in bronchial smooth muscles which leads to the intracellular release of calcium and this initiates bronchoconstriction. It is also well known that, histamine, a biogenic amine liberated by various stimuli such as degradation of mast cells causes bronchoconstriction along with other pathological conditions (inflammation). In addition, histamine is an important mediator of airway smooth muscle contraction, and the bronchial obstruction occurs via H_1 receptors. H_1 receptor blockade results in bronchodilation[12]. The presented data indicated a relaxant effect of HC extract on acetylcholine and histamine-contracted isolated guinea pig tracheal smooth muscles possibly via its cholinergic as well as histamine receptor antagonistic activity.

Present investigation shows bronchorelaxent action of HC extract at various extent and concentration dependent manner against acetylcholine as well as histamine induced bronchoconstriction in tracheal muscles of guinea pig. Inhibition of bronchoconstriction is considered as vital in the treatment of asthma as it interrupts further consequences caused by the same and may be helpful to provide relief from the asthmatic attack. The HC-ME also showed dose dependent percent bronchoprotection against acetylcholine and histamine aerosol induced brochospasm in guinea pigs. At 100 and 200 mg/kg the observed protection was 03.32 and 10.93 respectively. The result of percent bronchoprotection exhibited by HC-ME at 200 mg/kg was found to be insignificant when compared to control (Table 05). Observed bronchorelaxent effects by HC-ME might be contributed by its phytoprofile as it has been reported to exhibit triterpenoids and saponin. In addition water-soluble carbohydrate fraction as in *A. iwayomogi*, have been reported to exhibit significantly reduced pulmonary eosinophilia and Th2 cytokine expression in the lungs of B ALB/ c mice sensiized and challenged by OVA[13]. This also supports the fact that HC contents of carbohydrate and polysaccharides whereas later phytoconstituents have been reported to posses immounomodulatory and anti-inflammatory activity[14].

Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms[15].

To conclude, result obtained from above findings suggests the possible role of HC plant extract for the treatment of asthma. As HC extract inhibited bronchoconstriction induced by histamine or acetylcholine. HC extract also inhibit inflammation induced by carrageenan and egg albumin. Study related to detailed evaluation of extracts, fractions or specific active constituent(s) for complete understanding of mechanism and related effects is in progress at our research laboratories.

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