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Research Article

Preclinical Evaluation of Central Analgesic Activity along with Acute Anti-Inflammatory Effects of Methanolic Extract of *Asparagus Racemosus* using Experimental Animal Models

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ABSTRACT

Asparagus racemosus is traditionally used to treat pain and inflammatory disorders, yet scientific validation of its central analgesic and acute anti-inflammatory properties remains limited. This study evaluated the pharmacological potential of the methanolic extract of *A. racemosus* roots (MEAR) using rodent models. Dried roots were extracted via Soxhlet apparatus using methanol following petroleum ether defatting. Phytochemical screening was conducted using standard qualitative assays. MEAR (200 and 400 mg/kg, p.o.) was evaluated for central analgesic activity via hot plate and tail flick tests in albino rats, and acute anti-inflammatory activity via carrageenan-induced paw edema. Pentazocine and indomethacin served as standard controls. MEAR exhibited significant, dose-dependent central analgesic activity in both thermal models. Additionally, MEAR significantly suppressed carrageenan-induced paw edema, with the 400 mg/kg dose producing 33.3% maximum inhibition at 4 hours compared to 47.1% by indomethacin. MEAR demonstrates significant dose-dependent central analgesic and acute anti-inflammatory effects, supporting the traditional therapeutic use of *A. racemosus*. Further studies are warranted to isolate active constituents and elucidate their mechanisms.

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INTRODUCTION

Inflammation is a primary defence mechanism and protects body against toxic substances, allergens, infections and a number of harmful agents. Under various pathophysiological conditions, the inflammation process becomes uncontrolled and leads to chronic diseases. Generally, inflammation is regarded as a protective response intended to eliminate causes of injury such as noxious chemicals or microbial agents [1]. It is a complicated process that is mediated by variety of signals produced by leukocytes, macrophages and mast cells. Cyclooxygenases (COX) play a key role in the production of potent pro-inflammatory prostaglandins (PGs) [2]. Different synthetic drugs are used as anti-inflammatory agents. These include narcotics e.g., opioids or non-narcotics e.g., salicylates and corticosteroids e.g., hydrocortisone [3]. But the side effects of the currently available anti-inflammatory drugs present a major problem. Hence there is need to develop safe drugs [4]. Asian have written evidence for the use of natural products for various disease. Traditionally, natural products are approved as safe drugs in most of the societies as compare to allopathic medicines. A large number of phytochemicals isolated from natural sources have been used to treat inflammation and other pain-associated conditions. These phytochemicals showed low toxicity and higher therapeutic effect[5]. *Asparagus racemosus* is a commonly occurring plant from Liliaceae family. The common name of this plant is satmuli and the part used as therapeutic agent is the root. It normally grows at the height of 1 - 2 m, presenting needles like leaves and white flowers. Asparagus racemosus is used traditionally as carminative, antispasmodic, antidiarrheal and in dyspepsia and rheumatism. There is a need to prove its biological activities pharmacologically [6]. The aim of the present study was to evaluate the anti-inflammatory and analgesic activity of the methanolic extract of the root of Asparagus racemosus (MEAR) in carrageenan - induced paw oedema and thermal nociception modelsin albino rats respectively.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh roots of *Asparagus racemosus* were collected from the local region of Berhampur. The collected plant material was thoroughly washed with water to remove adhering debris and shade dried at room

temperature. A herbarium specimen was prepared and submitted for botanical identification and authentication by I. P. Padhy, Department of Pharmacognosy, Royal College of Pharmacy and Health Sciences. The authenticated plant material was stored in an airtight container for further experimental use.

Chemicals and Apparatus

Analytical grade methanol and petroleum ether were used as extraction solvents. The extraction and processing were carried out using a Soxhlet apparatus, heating mantle, condenser, round-bottom flask, beakers, glass rods, and rotary vacuum evaporator.

Preparation of Plant Extract (Successive Extraction)

The shade-dried roots were coarsely powdered and subjected to successive solvent extraction using a Soxhlet apparatus. Approximately 50 g of the powdered material was initially defatted with petroleum ether (60–80 °C). After defatting, the marc was dried and extracted with 500 mL of methanol by continuous hot percolation for 72 h. The obtained extract was filtered and concentrated under reduced pressure using a rotary evaporator at controlled temperature (50–60 °C). The concentrated extract was stored in a refrigerator until further analysis [7].

Preliminary Phytochemical Screening

The methanolic extract was subjected to qualitative phytochemical screening using standard methods to identify major secondary metabolites. The extract was tested for alkaloids, carbohydrates, glycosides, anthraquinones, saponins, gums and mucilage, proteins, tannins, phenolic compounds, triterpenoids, flavonoids, coumarins, steroids, sterols, and fixed oils. These tests were performed to determine the phytochemical composition of the extract [8].

Experimental Animals:

Healthy adult albino Wistar rats of either sex, weighing 150–200 g, were procured from the Animal House, Royal College of Pharmacy and Health Sciences, Berhampur. The animals were housed under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 55 ± 5%, and a 12 h light/12 h dark cycle) with free access to standard pellet diet and water ad libitum. The animals were acclimatized to the laboratory environment for 7 days before the commencement of the experiments. All experimental procedures were carried out in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA) and were

approved by the Institutional Animal Ethics Committee (IAEC).

Pharmacological Screening

Hot plate method:

Analgesic activity was tested in rat using the hot plate method. Twenty four numbers of rat were divided in to four groups (n=6). One group was given only distilled water and observed as a control. The second group was given pentazocine and treated as standard. Other 2 groups were given test drugs i.e. MEAR of 200 and 400 mg/kg respectively. All the drugs administered through p.o. route.

Albino rat was placed in aluminum hot plate kept at a temperature of 55 ± 0.5oC for a maximum time of 15 seconds. Reaction time was recorded when animals licked their paws or jumped. The responses were taken at different time interval i.e. 0, 30, 60, 120 and 180 minutes after administration of drugs. Cut off time in the absence of a response was 15 sec to prevent the animals from being burnt [9].

Tail flick method

The pre-screened animals were divided in to 4 groups. Total of 24 rats were divided in 4 groups of 6 each. One group was given only distilled water and observed as a control. The second group was given Pentazocine and treated as standard. Other 2 groups were given test drugs i.e. MEAR 200 and 400 mg/kg. All the drugs were given orally. The tail flick latency was assessed by Analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 6 amperes. Distance between the heat source and tail skin was 1.5 c.m. The site of application of the radiant heat in the tail was maintained at 2.5 cm measured from the root of the tail. The responses were taken at different time interval i.e. 0, 30, 60, 120 and 180 minutes after administration of drugs. The cutoff reaction time was fixed at 15 seconds to avoid tissue damage [10].

Carrageenan induced hind paw edema method

Twenty four numbers of rat were divided in to four groups (n=6). One group was given only distilled water and observed as a control. The second group was given indomethacin 5 mg/kg and treated as standard. Other 2 groups were given test drugs i.e. MEAR of 200 and 400 mg/kg respectively. Paw edema was induced, 30 minutes after the drug administration, by sub-planter injection of 0.1 ml of 1 % (w/v) freshly prepared suspension of carrageenan into the right hind paws of all rats. The volume of the injected paws and contra-lateral paws were measured at 0, 30, 60,

120, 180 and 240 minutes intervals by using Plethysmometer. The percentage inhibition of inflammatory edema in test and standard animal was calculated by: %inhibition = (Mean paw edema of control group – Mean paw edema of test group)/ Mean paw edema of control group × 100 [11].

Statistical analysis:

Data are expressed as mean ± SEM (standard error of mean) for five animals. The difference among means has been analyzed by students t test using Prism pad software. Differences were considered statistically significant at the value of probability less than 5% ($p<0.05$).

RESULTS

Percentage of yield (w/w), colour and consistency of different extracts:

The extract of *Asparagus racemosus* was filtered and then it was concentrated by distilling off the solvent to obtain the crude extract. The extractive values, color are tabulated below.

Table 1: Yield (%) of Extract of *Asparagus racemosus*

Sl. No.	Solvents	% yield (w/w)	Colour	Consistency
1	Petroleum-Ether (60–80)	3.87%	Darkish brown	Greasy mass
2	Methanol	11.95%	Brown	Dry powder

Phytochemical studies:

The Methanolic extract of *Asparagus racemosus* (MEAR) obtained from the above extraction process were analyzed for different phytoconstituents present in it by the method of qualitative phytochemical analysis. The results are depicted in table 2.

Hot plate methods

The analgesic property of Methanolic extract of *Asparagus racemosus* was studied on the experimental model of hot plate method in rat. The results obtained are tabulated in Table 2.

Table 2: Phytochemical screening of Methanolic extract of *Asparagus racemosus*

Sl. No.	Phytoconstituents	Presence / Absence
1	Alkaloid	+
2	Carbohydrate	+
3	Glycoside	+
4	Tannins	+
5	Protein & Amino acid	-
6	Gum and Mucilage	+
7	Flavones & Flavonoids	+
8	Saponins	+
9	Steroids & Sterols	-
10	Triterpenoids	+

Table 3: Mean response of hot plate method at various time interval.

Drug	DOSE	Mean Basal Time (Sec)	Mean response at different time (min)			
			30	60	120	180
Distilled water	0.5ml/kg	4.09±0.015	4.02±0.013	3.96±0.012	4.51±0.01	4.06±0.024
Pentazocin	10mg/kg	5.04±0.012	8.25±0.026	8.90 ± 0.025	10.94 ± 0.022	10.22 ± 0.021
MEAR	200 mg/kg	4.89 ± 0.017	7.09 ± 0.011	7.66 ± 0.029	8.81 ± 0.023	8.52 ± 0.011
MEAR	400 mg/kg	5.11 ± 0.007	8.03 ± 0.011	8.56 ± 0.011	9.77 ± 0.014	9.39 ± 0.011

The above table shows Pentazocine group have significant central analgesic effect as compared to distilled water treated group. The Methanolic extract of *Asparagus racemosus* is also showing significant analgesic effect in does dependent manner as that of standard drug.

Tail flick methods

The analgesic property of MEAR was studied on the experimental model of tail flick method in rat. The results obtained are tabulated below.

Table 4: Mean TFL of tail flick method at different time interval

Drug	DOSE	Mean Basal Time (Sec)	Mean response at different time (min)			
			30	60	120	180
Distilled water	0.5 ml/kg	4.25±0.019	4.03±0.014	4.66±0.015	4.16±0.013	4.01±0.007
Pentazocin	10 mg/kg	4.33±0.013	7.65±0.012	8.61±0.011	11.1±0.021	10.66±0.013
MEAR	200 mg/kg	4.63±0.01	7.12±0.009	7.54±0.009	8.85±0.009	8.33±0.011
MEAR	400 mg/kg	4.33±0.011	7.63±0.006	8.23±0.007	10.11±0.029	9.55±0.01

The above table shows Pentazocine group have significant central analgesic effect as compared to distilled water treated group. The Methanolic extract of *Asparagus racemosus* is also showing significant analgesic effect in does dependent manner as that of standard drug.

Acute inflammation study by carrageenan induced hind paw edema

The anti-inflammatory property of MEAR was studied on the experimental model of carrageenan induced hind paw edema in rat. About 0.1 ml of 0.6 % suspension of carrageenan was injected in the plantar region of right hind paw (Winter *et al*, 1962). The paw volume was measured by using Plethysmometer by mercury displacement method. The volume of paw edema was calculated to be the difference between the final paw volume and initial paw volume. Indomethacin was taken as standard drug. The results obtained are tabulated below.

Table 5: Mean paw volume at different time interval in acute inflammation study

Drug	DOSE	Initial paw volume	Mean paw volume at different time interval (in ml)				
			30min	1hr	2hrs	3hrs	4hrs
Distilled water	10 ml/kg	1.21±0.007	1.5±0.1	1.64±0.009	1.89±0.007	2.08±0.005	2.01±0.006
Indomethacin	5 mg/kg	1.24±0.005	1.43±0.005	1.61±0.03	1.62±0.007	1.7±0.005	1.64±0.005
MEAR	200 mg/kg	1.24±0.005	1.51±0.007	1.62±0.008	1.77±0.008	1.97±0.009	1.91±0.005
MEAR	400 mg/kg	1.24±0.004	1.45±0.008	1.53±0.004	1.68±0.007	1.82±0.006	1.74±0.005

Table 6: Mean paw edema at different time interval in acute inflammation study

Drug	DOSE	Mean paw edema at different time interval (in ml)				
		30min	1hr	2hrs	3rs	4hrs
Distilled water	10 ml/kg	0.29 ± 0.10	0.43 ± 0.009	0.68 ± 0.010	0.87 ± 0.007	0.80 ± 0.009
Indomethacin	5 mg/kg	0.19 ± 0.007	0.37 ± 0.031	0.38 ± 0.008	0.46 ± 0.009	0.40 ± 0.007
MEAR	200 mg/kg	0.27 ± 0.009	0.38 ± 0.009	0.53 ± 0.009	0.73 ± 0.011	0.67 ± 0.007
MEAR	400 mg/kg	0.21 ± 0.009	0.29 ± 0.009	0.44 ± 0.008	0.58 ± 0.008	0.50 ± 0.007

From the above tables No. 5 and No. 6 it is evident that, Indomethacin treated group have significant anti-inflammatory effect as compared to distilled water treated group. The Methanolic extract of *Asparagus racemosus* is also showing significant anti-inflammatory effect in does dependent manner as that of standard drug.

Table 7: Percentage inhibition of paw edema at different time interval

Groups	Groups % inhibition of paw edema at different time interval				
	30min	1hr	2hrs	3rs	4hrs
Indomethacin	5 mg/kg	34.5%	14.0%	44.1%	47.1%
MEAR	200 mg/kg	6.9%	11.6%	22.1%	16.1%
MEAR	400 mg/kg	27.6%	32.6%	35.3%	33.3%

CONCLUSION

The goal of the current study was to use well-established animal models to objectively assess the analgesic and anti-inflammatory qualities of *Asparagus racemosus*, often known as sweet basil. Bioactive substances including flavonoids, glycosides, tannins, and saponins with therapeutic potential in the treatment of pain and inflammation were detected by phytochemical screening of its methanolic extract. In both the hot plate and tail flick procedures, the extract showed strong dose dependent central analgesic action that was with that of the common medication pentazocine. Furthermore, the extract demonstrated significant anti-inflammatory efficacy in the carrageenan-induced paw edema model, particularly at the 400 mg/kg dosage, which was statistically equivalent to Indomethacin. These findings support the traditional application of *Asparagus racemosus* for inflammatory and pain disorders.

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