EVALUATION OF ANALGESIC AND ANTI INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF ASPARAGOUS RACEMOUS ROOTS IN RATS

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ABSTRACT

Asparagus racemous (Asparagaceae) is a common weed occurring throughout the globe. In traditional medicine its decoction has been used for treatment of many infectious and degenerative diseases. This work was therefore designed to assess the phytochemical constitution of *Asparagus racemous* dried roots extracts and to evaluate their analgesic and anti-inflammatory activity in rats. Fresh and crushed roots of *Asparagus racemous* were collected and then extracted with ethanol. The ethanolic extract at the doses of 100 mg/kg, 200 mg/kg body weight was subjected to evaluation of analgesic and anti-inflammatory activities in experimental animal models. Analgesic activity was evaluated by Hot-plate and tail –flick method in albino wistar rats; acute and chronic anti-inflammatory activity was evaluated by carrageenan-induced paw oedema and formalin-induced paw edema in Wistar albino rats. Diclofenac sodium and indomethacin were employed as reference drugs for analgesic and anti-inflammatory studies, respectively. In the present study, the ethanolic extract of *Asparagus racemous* demnstrated significant analgesic and anti-inflammatory activities in the tested models.

Keywords: *Asparagus racemous*, hot-plate, tail-flick, Carragenan-induced paw edema model, Formalin-induced paw edema model, Analgesic and anti-inflammatory activity.

Introdcution

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 [1]. Generally, inflammation is regarded as a protective response intended to eliminate causes of injury such as noxious chemicals or microbial agents [2]. 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) [3]. 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 [4]. But the side effects of the currently available anti-inflammatory drugs present a major problem. Hence there is need to develop safe drugs. Asian have written evidence for the use of natural products for various disease [5]. Traditionally, natural products are approved as safe drugs in most of the societies as compare to allopathic medicines [6].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 [7].

Asparagus racemosus is a commonly occurring plant in Punjab, Pakistan 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 [8].
Asparagus racemosus is used traditionally as carminative, antispasmodic, antidiarrheal and in dyspepsia and rheumatism [9]. There is a need to prove its biological activities pharmacologically. The aim of the present study was to evaluate the anti-inflammatory and analgesic activity of the aqueous methanolic extract of the root of Asparagus racemosus (AMEAC) in carrageenan and albumin- induced paw oedema and formalin-induced paw licking and acetic acid-induced abdominal writhing in albino mice respectively.

MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

DRUGS AND CHEMICALS

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Table No:1 Drugs and Chemicals

S.No	***Materials***	***Company Name***
1.	Carragean	Quali Kems Fine Chem Pvt, Ltd, Vadodara.
2.	Ethanol	ChangshuYangyuan Chemicals, China.
3.	Diclofenac	Sanofi India Ltd, Ankleshwar.
4.	Indomethacin	Samarth life sciences Pvt.Ltd

EXPERIMENTAL ANIMALS

Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 12hours of fasting from the day of Alloxan introduction. Animal studies had approval of IAEC.

PLANT MATERIAL COLLECTION

The roots of *asparagus racemous* was collected from the local market. The plant root material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

PREPARATION OF PLANT EXTRACTS:

3.5.1 Preparation of ethanolic Extract:

Fresh roots of *A. Racemous* were collected and washed under tap water. The root

extract used was prepared by taking 50gms of finely cut roots into 250ml beaker containing 200ml of ethanol. The contents were mixed well and then the mixture was boiled upto 50-70^0C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

SELECTION OF DOSE FOR ANIMAL STUDY

The dose considered for the experiment on rats was obtained from conversion ofhuman dose of *A. Racemous* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats. Hence the calculated dose for the rats (considering human dose 5 g/kg) is 100 and 200 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

PHARMACOLOGICAL EVALUATION

Preparation of extracts:

The ethanolic extracts of *Asparagus Racemous* suspended in water in presence of 3%v/v Tween-20 solution.

All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

ACUTE ORAL TOXICITY:

The acute oral toxicity of ethanolic extracts of *Asparagus Racemous* was determined by using Albino wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract upto 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed upto 7days for their mortality, behavioral and neurological profiles.

ASSESSMENT OF ANALGESIC ACTIVITY:

Table No: *2* Group Classification:

Group	**Treatment**	**Dose(mg/kg)**
Group 1	Normal control received distilled water	------
Group 2	Standard group received Diclofenac	10mg/kg
Group 3	Ethanolic extract of *A. Racemous*	100mg/kg
Group 4	Ethanolic extract of *A. Racemous*	200mg/kg

- **HOT-PLATE METHOD:**

Procedure:

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. Take the basal reaction-time by observing hind paw licking or jumping response in animals when placed on the hot- plate maintained at constant temperature (55^0C). Normally animals showed such response in 3-8 sec. a cut-off period of 15sec i.e observed to avoid damage to the paws. Inject the standard drug to group-2 and test drugs to group-3&4 and noted the reaction time of animals on the hot plate at 15, 30,
30 and 120min after the drug administration. Calculated the percentage increase in reaction time at each time interval.

- **TAIL-FLICK METHOD:**

Procedure: Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. The animals were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animals that held to withdraw its tail in 5 second was rejected from the study. Analgesia was assessed with a tail flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures were taken as 15, 30, 45 and 30 minutes interval and the reaction of the animals considered as the post – drug reaction time. A cut-off period of 10sec. was observed to prevents tissue damage of the tail of the animals.

ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY:

Table No: 3 Group Classification:

Group	**Treatment**	**Dose(mg/kg)**
Group 1	Normal control received distilled water	------
Group 2	Standard group received Indomethacin	10mg/kg
Group 3	Ethanolic extract of *A. Racemous*	100 mg/kg
Group 4	Ethanolic extract of *A. Racemous*	200 mg/kg

- **CARRAGEENAN-INDUCED PAW EDEMA METHOD**

Procedure:

Carrageenan-induced paw edema is a suitable experimental animal model for evaluating an anti edematous effect. Edema developed following injection of carrageenan serves as an index of acute inflammatory changes, was and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 3 hours.

Edema induced by carrageenan is believed to be biphasic: the first phase (1h) involves the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins.

The anti-inflammatory activity was determined using a carrageenan- induced paw edema model, Sixty Sprague-Dawley rats (200-240g) either sex, were randomly divided into 4 groups and fasted overnight before the experiment with free access to water. Treatments administered at their body weight to rats for one hour before subcutaneous injection of carrageenan (1% in NSS) into the plantar surface of the left hind paw.

After the carrageenan injection, the paw volumes were measured at 15, 30, 30&120min using a Plethysmometer (Dolphin, India). The difference between the intial and subsequent readings gave the actual edema volumeEdema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation:

% inhibition of edema = 100 (1-Vt/Vc), Where Vc is the edema volume in the control group and

Vt is the edema volume in tested group.

- **FORMALDEHYDE INDUCED PAW EDEMA METHOD**

Procedure:

Inflammation was induced by injection of 0.1ml of freshely prepared Formaldehyde (3%) underneath the plantar tissue of right hind paw. the test drug was administered consecutively for seven days to all groups. on seventh day, after 1h of drug administration, paw edema of the rat was induced by subplantar injection of formaldehyde solution. The paw volume was determined at 0h and at 3, 3, 24 and 48h after formaldehyde injection.

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparision had made. i.e.

1. Normal control Vs All treated groups.

Differences between groups were considered significant at P<0.001 and *P* <0.05 levels.

RESULTS AND DISCUSSION

ACUTE TOXICITY STUDIES:

Acute toxicity studies revealed that the ethanolic extracts of *Asparagus racemous* were safe up to 1000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

EVALUATION OF ANALGESIC ACTIVITY

HOT PLATE METHOD:

The analgesic activity of EEAR was assessed using Hot plate method in Swiss albino rats was illustrated in Table. EEAR showed significant analgesic activity at 100 and 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both doses has showed maximum analgesic activity at reaction time is 12 and 5 sec respectively and the standard drug Diclofinacreaction time is 10 sec. **Table No: 4 Effect of extracts of *Asparagus racemous* on Analgesic activity.**

Groups	**Dose (mg/ kg)**	**Basal reaction time(sec)**		**After drug administration(sec)**							
		0min		**15min**		**30min**		**60min**		**120min**	
		Paw licking(P)	**Jumping(J)**	**P**	**J**	**P**	**J**	**P**	**J**	**P**	**J**
Control	**10**	-	9	4	6	5	3	3	-	5	-
Standard	**40**	9	-	10	-	6	-	14	-	-	10
EEAR	**100**	14	-	5	-	12	-	4.2	-	12	-
EEAR	**200**	12	10	4	10	-	10	-	12	-	5

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

TAIL-FLICK METHOD:

The analgesic activity of EEAR was assessed using Tail-Flick method in Swiss albino rats was illustrated in Table. EEAR showed significant analgesic activity at 100 and 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both two doses showed maximum analgesic activity at reaction time is 14 and 12 sec respectively and the standard drug Diclofenac reaction time is 13 sec.

Table No: 5 Tail Flick Method.

S.No	**Treatment**	**Dose (mg/ kg)**	**Reaction Time(sec)**				
			30min	**45min**	**60min**	**4.15min**	**90min**
1.	control	-	6.43±0.13	6.35±0.10	6.20±0.09	6.32±0.09	6.25±0.06
2.	standard	10	9.4±0.14	11.62±0.04	13.52±0.13	13.42±0.05	11.4±0.04

3.	EEAR	100	9.4±0.10	9.64±0.15	12.65±0.04	14.25±0.05	12.4±0.04
4.	EEAR	200	9.6±0.06	9.4±0.12	10.04±0.14	12.64±0.03	10.4±0.13

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

EVALUATION OF ANTI INFLAMMATORY ACTIVITY

CARRAGENAN INDUCED PAW OEDEMA IN RATS

In carragenan induced paw oedema activity, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in Table No: ---. The tests compounds are compared with diclofenac as a standard at a dose of 40mg/kg for anti-inflammatory activity. Presently diclofenac showed 20% inhibition of inflammation at 2 hours when compared to control.

Ethanolic extracts of *asparagus racemous* roots(100 and 200 mg/kg) shown significant inhibition of inflammation with 30% and 10% respectively at 2 hours when compared with control. The results of test compounds were found to be statistically significant at value P<0.05.

Table No: 6 Effect of extracts of *Asparagus racemous* on paw oedema volume.

GROUP S	**Dose (mg/kg)**	**Change in paw volume (ml) mean±SEM & Percentage inhibition**									
		0min		**15min**		**30 min**		**60 min**		**120min**	
		R	**L**	**R**	**L**	**R**	**L**	**R**	**L**	**R**	**L**
Control	--	0.2±0.1	0.3±0.2	0.2±0.1	0.5±0.3	0.2±0.1	0.3±0.2	0.2±0.1	0.3±0.1	0.2±0.1	0.3±0.2
Std (Diclofe	**10**	0.2±0.	0.3±0.	0.2±0.1	0.4±0.2	0.2±0.1	0.3±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1

nac Sodium)		1	2								
EEAR	**100**	0.3 ±0. 1	0.5 ±0. 2	0.3± 0.2	0.5± 0.2	0.2± 0.1	0.4± 0.2	0.1± 0.2	0.3± 0.1	0.2± 0.1	0.2± 0.1
EEAR	**200**	0.2 ±0. 1	0.3 ±0. 1	0.2± 0.1	0.3± 0.2	-	0.2± 0.1	0.2± 0.1	0.2± 0.1	0.1± 0.2	0.2± 0.1

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

FORMALIN INDUCED PAW EDEMA

The results of anti-inflammatory activity of ethanolic extract of *Asparagus racemous* roots in formaldehyde induced paw edema is shown in table---. Injection of formaldehyde subcutaneously into hind paw of rats produces localized inflammation. The administration of EEAR-100 and 200mg and Indomethacin- 10mg for 4.1days successfully inhibited edema induced by formaldehyde. EEAR- 100 and 200 group showed maximum decrease in paw volume at 3h ($P<0.05$ and $P<0.01$) and decreased in paw volume at 44.2h.

Table No:7 Anti-inflammatory activity of ethanolic extract of *Asparagus racemous* roots in formaldehyde induced rat paw edema.

S.No	**Treatme nt**	**Do se Mg**	**% increase in paw volume**					
			After 3h		**After 24h**		**After 44.2h**	
			Vol increas	**% change**	**Vol increas**	**% change**	**Vol increase**	**% change**

		/kg	**e**		**e**			
1.	Control	--	4.12±0.06	-	4.4±0.04	-	5.0±0.02	-
2.	Standard	10	2.59±0.03	56.4%	2.43	55.2%	2.12	42.4%
3.	EEAR	100	2.01±0.03	44.2%	2.21	50.2%	2.13	42.6%
4.	EEAR	200	2.33±0.03	56.5%	2.24	50.9%	2.02	40.4%

DISCUSSION

ANALGESIC ACTIVITY HOTPLATE METHOD:

The extracts increased reaction latency to thermal pain induced by the hot plate method in rats, which is a specific central antinociceptive test. Inhibition of histamine or kinin pathway may reduce pain. The results of the present study also showed that extract exhibited a comparable magnitude of antinociceptive activity in hot plate method of pain which suggested that the phytochemical constituents are responsible for the analgesic effect. The results of the present study indicated that the ethanolic extracts of *Asparagus racemous* might contain constituents capable of relieving or modifying responses to pain caused by either thermal or chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms.

TAIL FLICKMETHOD:

the extracts showed significant analgesic activity at all tested dose levels. In tail flick method, the ethanolic extracts of *Asparagus racemous* roots at a dose of 100 and 200 mg/kg showed significant activity. The results showed significant analgesic activity against thermal stimuli. The analgesic studies revealed that the methanolic extract of *Asparagus racemous* roots exhibited potent analgesic (central analgesic activity) effect against thermal noxious stimuli and also revealed that the extract shows dose dependent analgesic effect.

ANTI-INFLAMMATORY ACTIVITY

CARRAGEENAN INDUCED RAT PAW EDEMA MODEL:

It is believed that current anti-inflammatory drugs such as opioids and non- steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, search for other alternatives became necessary and imperative. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Asparagus racemous* roots using carragenan induced rat paw edema for anti-inflammatory models.

Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti- inflammatory activity. Carrageenan induced rat paw edema is a suitable model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. Carrageenan-induced hind paw edema in rat is a

biphasic event. The early phase (90 - 14.20 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (24.10–
360 min) is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome. The aqueous and alcoholic extracts of *Asparagus racemous* roots inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX- dependent mechanism, thus, it is suggested that the AQEEU and ALEEU may possess arachidonate COX inhibitory property.

FORMALIN INDUCED RAT PAW EDEMA MODEL:

Formalin induced paw edema in rats is one of the most suitable test procedure to screen the acute inflammation and it is believed to be a biphasic event. The anti inflammatory effects of triterpenes have been attributed to various mechanisms including inhibition of lipoxygenase and cycloxygenase activities.

Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. LPO level was increased during inflammation. Administration of formalin produced an elevated level of LPO, which may due to the free radicals and is responsible for damaging cell membranes there by further intensifying inflammatory damage. The inflammatory tissue damages could be due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites. Hence, in the present study, the concentration of LPO was found to be higher in formalin induced rats. On treatment with the *Asparagus racemous* at the dose level of 100, 200 mg/kg bw, the LPO level was significantly decreased.

Formalin induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of relative composition of various constituents of connective tissue such as muco polysaccharides, glyco protein, hexosamine and hydroxy proline, sialic acid. Hence the levels of hexosamine and hydroxyproline were found to be higher in formalin induced rats. Pretreatment of Asparagus racemous inhibited the accmulation of hydyoxy proline and hexosamine in edematous tissue of formalin induced rats.

CONCLUSION

Asparagus racemous is a plant and it has anti-diabetic, anti-bacterial, anti- microbial, anti-fungal, anti-oxidant and CNS activities. Among these studies it could be concluded that roots of *Asparagus racemous*have shown great potential of anti- inflammatory and Analgesic activity. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific drugs.

ANTI-INFLAMMATORY ACTIVITY:

In the present study it has concluded that the ethanolic extracts of *Asparagus racemous* have anti-inflammatory activity in carragenan-induced and Formalin induced paw edema in rats. This extracts has showed that decrease in paw edema volume when compared to control and standard drugs. Therefore the anti-inflammatory effects observed in this study may be due to the activity of one or a combination of some of the identified constituents. It may suggest that the inhibitory effect of the constituents in the extract on edema formation is probably due to inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carragenen induced paw edema test is effectively controlled with the arachidonate COX inhibitors due to its COX-dependent mechanism.

ANALGESIC ACTIVITY:

In the Present study it has concluded that ethanolic extracts of *Asparagus racemous* have an analgesic activtiy in both Hotplate and Tail-flick method in rats. This extracts has

showed that increase mean latency to thermal pain. The presence of some chemical constituents contains a capable of relieving or modifying responses to pain caused by inhibition of histamine or kinnin pathway.

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