COMPARISON OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF ASPARAGUS RACEMOSUS WITH ASPIRIN

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ABSTRACT

Objectives:

To Compare Analgesic and Anti-inflammatory activity of Ethanolic Extract of Asparagus racemosus with Aspirin.

Materials and Methods:

Analgesic method – 18 Albino mice of either sex weighing about 20-25 gm were randomly divided in to 3 groups of 6 animals each. The control group, Standard and test group were given distilled water, Aspirin (100mg/kg) and Ethanolic extract of Asparagus racemosus (200mg/kg) respectively. Drugs were given orally after overnight fasting. Analgesic activity is evaluated before drug administration, after 2 hrs and 4 hrs of drug administration by Tail clip method. A wash out period of 15 days is given and the same group was utilized for evaluation of analgesic activity by hot plate method.

Anti-inflammatory activity – 18 Albino rats of either sex weighing about 175-225 gm were randomly divided in to 3 groups of 6 animals each. The control group, Standard and test group were given distilled water, Aspirin (300mg/kg) and Ethanolic extract of Asparagus racemosus (1600mg/kg) respectively. Drugs were given orally after overnight fasting. Anti-inflammatory activity is evaluated by measuring foot paw volume by using plethysmograph before administration of 1% carrageenan and 3 hrs after drug and 1% carrageenan administration.

Results:

The observations from Analgesic activity of the extract showed, the mean reaction time(In Tail clip and Hot plate method) in the test group after 2nd and 4th hour of drug administration was statistically significant ($P<0.01$, $P<0.001$) and it was comparable with Aspirin. Observations from Anti-inflammatory activity of the extract showed, there was 61.11% of inhibition of edema in the test group and it was comparable with the standard which had 69.45% of inhibition of edema.

Conclusions:

Ethanolic extract of Asparagus racemosus has analgesic and anti-inflammatory activity which was comparable with Aspirin.

Key Words : Plethysmograph, Carrageenan, Asparagus racemosus

INTRODUCTION:

Pain is one of nature's earliest sign of morbidity and it stands preeminent among all the sensory experiences by which human judge the existence of diseases within them. Pain remains the primary reason for which patient seeks the medical advice. Inflammation is fundamentally a protective response which helps the organism to get rid of microbes, toxins, necrotic cells and tissues. Chemical mediators of pain and inflammation are histamine, serotonin, prostaglandins, leukotrienes and cytokines[1].

Pain and inflammation are commonly treated with Non Steroidal Anti-inflammatory drugs (NSAIDS). Opioid analgesics are used in the treatment of severe visceral, postoperative, ischemic, traumatic and cancer pain. But these have adverse effects like gastric mucosal damage, respiratory alkalosis, increase in bleeding tendency, hypersensitivity reactions, renal disorders, hepatic damage etc2. So a search of drugs with good therapeutic effect and fewer side effects is going on.

The planet earth has been blessed with vast majority of flora and fauna. Most of these remain uninvestigated in the search for biomolecules with specialized structures and target specificity. Plants are potential sources of medicine. The various natural compounds present in plants act on all systems of the body and have high therapeutic activity.

The Asparagus genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant. About 300 species of Asparagus are known to occur in the world. Asparagus racemosus (shatavari) was recommended successfully by Ayurvedic practitioners for the prevention and treatment of gastric ulcers, galactogogue,

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antihepatotoxic and immunomodulatory activites[3]. Literature reveals that it also has analgesic and anti-inflammatory activites[4]. In the present study an attempt has been made to evaluate the analgesic and anti-inflammatory activity of Asparagus racemosus by comparing with the standard drug aspirin using albino mice and albino rat.

Materials and Methods:

Plant Material:

The roots of Asparagus racemosus were collected from Western Ghats. The root identification was confirmed by the Professor and HOD, Department of Botany, Madurai Kamaraj University, Madurai. The root was shade dried and powdered.

Preparation of Ethanolic Extract:

The dried and powdered root material of Asparagus racemosus (100gm) was added with 1L of absolute alcohol and the extract was obtained by Soxhlet method5.

Animals:

18 Albino rats and 18 Albino mice of either sex were obtained from the central animal house, Madurai medical college, Madurai. The Animals were housed in standard cages and acclimatized for a period of 15 days. The mice and rats were maintained in standard pelleted diet and water ad libitum. Approval for the study was obtained from the Institutional Animal Ethical committee, Madurai Medical College, Madurai.

Drugs and Chemicals:

Aspirin – Acetyl salicylic acid powder is obtained from Unichem laboratory, Mumbai. 1% Carrageenan – They are linear sulfated polysaccharides obtained from red sea weed (Chondrus crispus). When injected in to the tissues, it induces inflammation by activation of interleukin pathway[6]. The chemical was obtained from Kumar organic products limited, Bangalore. 1% solution was made by dissolving 1 gm of carrageenan in 100 ml of normal saline.

Equipments:

Tail Clip[7] – Bull dog clamp with thin rubber sleeve was used to produce noxious stimuli by mechanical pressure (Tail Compression).

Eddy's Hot Plate[8] – It was designed to study the analgesic effect of drug at different temperature. The temperature was monitored on a galvanometer. In this method heat was used as source of pain.

Plethysmograph – It is an apparatus containing mercury. The apparatus has 'U' shaped limb, that is connected to the vertical limb through a knob. The knob is adjusted to ensure adequate height of mercury column in the 'U' shaped limb. The mercury displacement due to dipping of the paw was read from the scale attached to the mercury column.

Drug Administration:

The Drugs and chemicals used in this study were orally administered with the help of oral feeding tube. (18-20 gauge needle for mice, 15-16 gauge needle for rat[9])

Evaluation of Analgesic activity:

Analgesic activity is evaluated by Haffner's tail clip method demonstrated by Binachi and Franceschini and Eddy's hot plate method. In Haffner's tail clip method 18 albino mice of 20-25 gm were selected. A Tail clip was applied to the base of the mouse tail. The reaction time made by the animal to the noxious stimuli was observed. Those animals not responding to the painful stimuli within 10 sec (cut off time) were not included for the study8. The responsive animals were randomly grouped in to 3 groups of 6 animals each and administered with Ethanolic extract of Asparagus racemosus (200mg/kg) for the test group. Aspirin (100mg/kg) was used as a positive control and the negative control received only the distilled water. The drugs were administered after overnight fasting. Reaction time was observed before and after 2 and 4 hr of drug administration and the results were tabulated (Table-1).

In Eddy's hot plate method 18 albino mice of 20-25gm were utilized after a wash out period of 15 days. The animals were kept over Eddy's hot plate maintained at 50-55°c(for 30sec). Reaction time made by the animal like licking of the paws or jump response was observed. The responsive animals were grouped in to 3 groups of 6 animals each. The treatment was given similar to tail clip method. Reaction time was observed before and after 2 and 4 hr of drug administration and the results were tabulated (Table-2).

Evaluation of Anti-inflammatory activity:

Anti-inflammatory activity is evaluated by carrageenan induced rat paw edema method. In this method 18 adult albino rats of either sex were randomly divided into 3 groups of 6 animals each and administered with Ethanolic extract of Asparagus racemosus (1600mg/kg) for the test group, Aspirin (300mg/kg) to the standard group and the control group received only distilled water. The drugs

were administered after overnight fasting. Immediately after drug administration 0.1 ml of 1% Carrageenan was administered subplantarly to the left hind paw. A mark was made in the ankle joint to ensure the dipping of the rat paw to the same level in the plethysmograph every time. Paw volume was measured before and 3 hrs after carrageenan administration. Mean paw volume in the control, test and standard group was calculated by the formula-percentage of inhibition of edema. The results were analyzed and the percentage of inhibition of edema was compared (Table-3).

Statistical Analysis:

The results of tail clip and hot plate method were analyzed statistically by students unpaired 't' test. The results of anti-inflammatory activity were analyzed by using the following formula.

Percentage of inhibition of edema = Vc-Vt/VcX100.

Where Vc – Mean edema in the control group.

Vt – Mean edema in the treatment group (standard and test)10.

Results:

Tail clip Method:

The mean reaction time for the distilled water group after 2 and 4 hrs of drug administration was 4±2.61 sec and 4.5±2.81 respectively. Hence it had no significant analgesic activity. The mean reaction time of the Aspirin group after 2 and 4 hrs of drug administration was 18±6.23sec and 22.67±5.72 respectively. It had statistically significant analgesic activity ($P<0.01$) in comparison with control group. The mean reaction time of the Ethanolic extract group after 2 and 4 hrs of drug administration was 24.5±8.98sec and 26.67±8.17sec respectively. It also had statistically significant analgesic activity ($P<0.001$) in comparison with control group (Table-1).

Hot plate Method:

The mean reaction time for the distilled water group after 2 and 4 hrs of drug administration was 4.5±1.38 sec and 4±1.26 respectively. Hence it had no significant analgesic activity. The mean reaction time of the Aspirin group after 2 and 4 hrs of drug administration was 15±2.19sec and 12.5±3.21 respectively. It had statistically significant analgesic activity ($P<0.01$) in comparison with control group. The mean reaction time of the Ethanolic extract group after 2 and 4 hrs of drug administration was 19.33±8.91sec and 18.83±8.77sec respectively. It also had statistically significant analgesic activity ($P<0.001$) in comparison with control group (Table-2).

Anti-inflammatory activity:

The mean paw volume of the distilled water group was 1.08 ±0.08mm. There was no significant inhibition of inflammation in the distilled water group. The mean paw volume in Aspirin group was 0.33±1.005mm. It had 69.45% of inhibition of inflammation. The mean paw volume in the Ethanolic extract group was 0.42±0.7043mm and it had 61.11% of inhibition of inflammation (Table-3).

Discussion:

The observations from the present study revealed that mean reaction time in Haffner's tail clip method and Eddy's Hot plate method are prolonged in Standard and Test groups compared to the control. The prolongation of reaction time is due to analgesic activity of the standard and test drug. Statistical analysis also revealed significant analgesic activity in the standard and test group. In the study of Anti-inflammatory activity, it was found that the % of inflammation in the standard and test groups was significantly reduced when compared with the control. This was due to anti-inflammatory activity of standard drug and the extract (Asparagus racemosus).

Asparagus racemosus has steroidal saponins, sapogenins, isoflavins, tannins, alkaloids(asparagamine) as essential constituents[11]. The steroidal saponins and isoflavins resemble hormones. This constituent may be responsible for its anti-inflammatory activity. The extract was safe even at the dose of 64gm/kg[12]. Being a gastro protective and galactogogue[13] the extract can be used as a safe analgesic and anti-inflammatory even in hepatic diseases and pregnancy. Since it has bronchodilatory property and devoid of any hypersensitivity reactions[14], it can safely used in asthmatics as bronchodilator and anti-inflammatory agent. It has been called as nervine tonic and used in the nervous disorders[15].Hence the extract can also be used in the treatment of nervous disorders with pain.

Conclusion:

The results of the present study indicate that the Ethanolic extract of Asparagus racemosus had statistically significant analgesic and anti-inflammatory activity and it was comparable with Aspirin. Hence further studies on Asparagus racemosus directed towards the isolation and identification of the active constituent of the extract may

provide an opportunity for the development of a novel class of agent for the treatment of pain and inflammation with gastro, hepato and bronchoprotective effect and less toxicity.

Table-1: Analgesic activity of Asparagus racemosus in comparison with Aspirin by Tail clip method

Groups	Reaction time(sec) Mean±S.D	
	2 hrs	4 hrs
Control	4±2.60	4.5±2.81
Standard(Aspirin)	18±6.23 ??	22.67±5.72 ??
Test(Ethanolic extract of Asparagus racemosus)	24.5±8.98 ???	26.67±17 ???

?? - P<0.01 ??? - P<0.001

Table-2: Analgesic activity of Asparagus racemosus in comparison with Aspirin by Eddy's hot plate method

Groups	Reaction time(sec) Mean±S.D	
	2 hrs	4 hrs
Control	4.5±1.38	4±1.26
Standard(Aspirin)	15±2.19 ??	12.5±3.21 ??
Test(Ethanolic extract of Asparagus racemosus)	19.33±8.91 ???	18.83±8.77 ???

?? - P<0.01 ??? - P<0.001

Table-3: Anti-inflammatory activity of Asparagus racemosus

Groups	Mean paw volume ±S.D	Mean% of Inflammation	Mean % of inhibition of inflammation
Control	1.08±0.08	100	0
Standard(Aspirin)	0.33±0.105	30.55	69.45
Test(Asparagus racemosus)	0.42±0.07	38.89	61.11

References:

1. Vinaykumar et al. Acute and Chronic inflammation. In: Vinaykumar et al. Robbins and Cotran Pathologic basis of Disease, 7th edition. India: Elsevier Saunders; 2005. P .48-85.
2. HL Sharma and KK Sharma. Nonsteroidal Anti-inflammatory Agents, Antirheumatic Drugs and Antigout Drugs. In: HL Sharma and KK Sharma. Principles of Pharmacology, 1st edition. Hyderabad: Paras Medical Publisher; 2007. P .367-384.
3. Goyal RK singh J. Asparagus racemosus – an update. In: Indian Journal of Medical Sciences, 2003; 57: p. 408-414.
4. Nadkarni AK. Asparagus racemosus – wild. In: Nadkarni AK. Indian Materia Medica, 1st edition. Bombay: Popular Book depot; 1954. I: p .153-155.
5. William Charles Evans. Quality Control. In: William Charles Evans. Trease and Evan's Pharmacogonosy, 15th edition. Philadelphia: W.B. Saunders; 2002. p .95-100.
6. Borthakur A. et al. Am J Physiol Gastrointest Liver Physiol (2007) 292:G829–G838
7. M.N.Ghosh. Some Common Evaluation Techniques. In M.N.Ghosh. Fundamentals of Experimental Pharmacology, 3rd edition. Kolkata, India: Hilton & Company;2005. p .175-179.
8. S.K.Kulkarni. Experiments on intact preparations. In: S.K.Kulkarni. Practical pharmacology and clinical pharmacy, 1st edition. New Delhi, India: Vallabh Publications; 2008. p .136-166
9. Alwin F Moreland. Collection, Withdrawal and Infusion techniques. In: Alwin F. Moreland. Methods of Animal Experimentation, 1st edition. New York: Academic Press; 1965. p .33-35
10. Robert Arnold Turner. Analgesics, Antiinflammatory agents. In: Robert Arnold Turner. Screening Methods in Pharmacometrics, 1st edition. New York Academic press; 1965. p .100-164.
11. P.K.Warrier et al. Asparagus racemosus wild. In: P.K. Warrier et al. Indian Medicinal Plants, 1st edition, Chennai, India: Orient Longman; 1996. I: p .218-223.
12. Sharma PC et al. Data base on Medicinal plants used in Ayurveda. In: Documentation and Publication Division, Central Council for research in Ayurveda & Siddha; 2000. I; p .418-430
13. Narendranath et al. Effect of herbal galactogogue a pharmacological and clinical observation. Med surg1986; p .19-22.
14. Carmen Tamayo. Clinical Trials of Herbal Medicines. In: Pulok k.Mukerjee and Robert Verpoorte. GMP for Botanicals, 1st edition. New Delhi, India: Business Horizons; 2003 p .331-338.
15. K.S.Krishna Marg.Asparagus. In: K.S.Krishna Marg. The Wealth of India, 1st edition. New Delhi, India: NISCAR press; 2004. I, A-Ci: p .101-102.