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Evaluation of Anti-Inflammatory and Analgesic Activity on *Actiniopteris dichotoma bedd*

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ABSTRACT
The present study was aimed to find out acute toxicity and investigate the analgesic and anti-inflammatory effect of the methanolic extract of *Actiniopteris dichotoma bedd* in rats. The acute toxicity study showed that animals fed by oral gavages tolerated the limit dose of 5000 mg/kg body weight of methanolic extract of *Actiniopteris dichotoma bedd*. There were no visible signs of acute toxicity during the 14 days of observation. *Actiniopteris dichotoma bedd* was evaluated carrageenan induced paw edema and histamine induced paw edema (250mg/kg and 500 mg/kg). At the 250 mg/kg dose of methanolic extract of *Actinopteris dichotoma* showed inhibition ($P < 0.05$) and the maximum inhibition of methanolic extract at 500 mg/kg at the time of 180 min and 240 min ($P < 0.01$). Methanolic extract of *Actiniopteris dichotoma bedd* **(**250mg/kg and 500 mg/kg) for its analgesic activity by hot plate method and tail immersion method. It showed Significant value from control $P < 0.05$*, $P < 0.01$**, $P < 0.001$***.

Keywords: Methanolic extract, *Actiniopteris dichotoma bedd,* anti-inflammatory, analgesic.

Introduction:

India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries.[1]

Actiniopteris dichotoma bedd have been used in the treatment of various types of diseases by the tribal from long times.whole part of this plant is useful. It is Small fern about 4- inch high very much like a miniature palm; cuadex oblique stipes densely tufted, 2-4 inch high, scaly; fronds like leave of a small palm, fan-like; segments dichotomous, radiating narrowly-linear, rush-like in texture, Veins not many and parallel with indistinct midrib; segments of fertile frond longer than the segments of barren ones. It is found throughout of India and very common in the lower hills of Atta paddy, upto 600m in Nilgiris and the rocky hillsides of Western Ghats. It is found practically in all the districts of the State on slopes of hills specially on the northern aspect. Plant pacifies vitiated kapha, pitta, diarrhea, dysentery, worm infestations, skin discoloration, skin diseases, diabetes and fever.

Inflammation is one of the common denominators of disease. Every chronic disease is an inflammatory disease. No matter what so-called disease you have, from cancer to the common cold, inflammation is a major part of your problem. Learning how to prevent and reverse inflammation will go a long way toward preventing and reversing almost all disease, as well as slowing the aging process, keeping us healthy, biologically young and vigorous for a lifetime.[2]

Pain is not only an unpleasant sensation, but a complex sensory modality essential for survival. There are rare cases of people with no pain sensation.[3] The International Association for Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. It is to be noted that pain is not just a physical sensation. It is also an emotional experience.

MATERIAL AND METHODS:

Collection of crude drug

The whole plant was obtained from Chitrakoot region, District Satna, Madhya Pradesh. The plant was authenticated by Dr. H.B. Singh, H.O.D. of National Institute of Science Communication and Information Resources (NISCAIR), near Pusa gate, New Delhi, India.

Preparation of extract

The whole plant was dried and powdered. A fine coarse powder was obtained which was sieved through #40 to obtain uniformity. The powered obtained was successively extracted in petroleum ether, chloroform, acetone, methanol and distilled water. Continues soxhlet extraction method was used, the powder of crude drug was packed in a thimble made whatman filter paper and then inserted in to the extractor. Each batch extracted for about 35 cycles.

The extracts were then made to powder by using rotary evaporator under reduced pressure. Crude drug of *Actiniopteris dichotoma* yielded 0.9%, 1.5%, 2.4%, 3.4% and 4.6 % w/w powered extract with petroleum ether, chloroform, acetone, methanol and distilled water respectively. When the extraction was completed, the extractant concentrated under vacuum, for large volumes and by heating at low temperature. Aqueous extracts were generally freeze-dried and stored at 20°C as this low temperature reduces the degradation of the bioactive natural product.

Preliminary phytochemical analysis

All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents. The plant extracts were subjected to qualitative tests for the identification of the phytoconstituents present in it viz., alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins and free amino acids, gums & mucilage's, Flavonoids, lignin and saponin.

Experimental animals

Male wistar albino rats weighing 150-180 gm were purchased from DRDE, Gwalior, and M.P. the animals were housed in polypropylene cages and maintained in control temperature (26+ 20C). and light cycle (12 h light and 12 h dark); they were fed with modern scientific animal feed, jayendraganj, Gwalior. Water was supplied ad libitum. Initial body weight of each animal was recorded. Ethical clearance for the use of animal was obtained from CPCSEA and registration No. is 891/po/ac/05/CPCSEA.

Procedure of Acute Oral Toxicity

The acute oral toxicity of the crude ethanolic extracts of *Actiniopteris dichotoma* was evaluated in mice using the procedures described by Organization for Economic Co-operation and Development 423 guidelines.[4]

A total of 18 female animals were divided into three dosage groups with 6 animals per dose. The control group was given 10 ml/kg of normal saline. The second and third groups were given with a single dose of 2000 mg/kg and 5000 mg/kg of *Actiniopteris dichotoma*, respectively. Gavage dosing was performed using a curved, ball-tipped intubation needle affixed to a 5 ml syringe. All solutions were prepared just prior to dosing and were kept chilled and tightly capped. Body weight, food, and water consumption were monitored daily. Animals were fasted approximately 12 hours prior to dosing. Following administration of a single dose of herbal preparation, the animals were observed for behavioural changes and general toxicity signs. Results were recorded for the first 30 minutes and at hourly intervals for the next 24 hours.

Experimental design

ANTI-INFLAMMATORY ACTIVITY-

***Carrageenan induced paw edema model*:**

The anti-inflammatory activity of the extract was determined using carrageenan induced rat paw edema assay. Wister Albino rats of either sex weighing 150-200 grams were divided into five groups of six animals each.

The dosage of the drugs administered to the different groups was as follows.

Group I - Negative Control served as normal saline 5 ml/kg

Group II- Positive control served as Carrageenan 0.1 ml of 1% W/V

Group III- Extract of *Actiniopteris dichotoma* was given at 250 mg/kg

Group IV - Extract of *Actiniopteris dichotoma* was given at 500 mg/kg

Group V - Indomethacin served as standard, dose at 5mg/kg.

All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug. After one hour of the administration of the methanolic extract of *Actiniopteris dichotoma and indomethacin* drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw. The paw volume of the rats were measured in the digital plethysmograph, at the end of 0 min., 1 hrs,2 hrs,3 hrs,4 hrs,5 hrs. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation.[5]

Histamine induced hind paw edema :

The anti-inflammatory activity of the methanolic extract of *Actiniopteris dichotoma* was determined using histamine induced rat paw edema assay. Inflammation was induced by injection of 0.1ml of freshly prepared histamine (1%) aqeous suspension normal saline under meath in the planter tissue of right hind paw of rats. The drug treatment and paw value was measured in a similar manner to that of carrageenan induced paw volume model. Wister Albino rats of either sex weighing 150-200 grams were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows-

Group I - Negative Control served as normal saline 5 ml/kg

Group II- Positive control served as histamine 0.1 ml

Group III - Extract of *Actiniopteris dichotoma* was given at 250 mg/kg

Group IV - Extract of *Actiniopteris dichotoma* was given at 500 mg/kg

Group V –Standard drug Indomethacin served as 5 mg/kg

Extract of plant was administered orally. Indomethacin served as the reference standard anti-inflammatory drug. After one hour of the administration of the drugs, histamine solution in normal saline was injected into the sub plantar tissue of the left hind paw of rats. The paw volume of the rats were measured by the digital

plethysmograph, at the end of 0 min., 1 hrs,2 hrs, 3 hrs, 4 hrs, 5 hrs. Mean increase in Paw volume and percentage of antiinflammatory activity were calculated. The results were statistically analysed by analysis of variance.[6]

ANALGESIC ACTIVITY

Hot Plate Test Method:

Hot plate method was employed to evaluate the analgesic activity of the extract. In this method heat is used as a source of pain. Animals were individually placed on a hot plate maintain at constant temperature (55 ± 0.1°C) and the reaction of animal, such as paw licking or jump response was taken at the end point. Normally animal show such response in 6 to 8 second. A cut of period 15 second is observed to avoid damage to the paws.[7] Experimental animals (albino mice) were divided into four groups designated as group-I, group-II, group-III and group-IV consisting of six mice in each group. The different groups were treated as follows:

Group I - Control served as normal saline 5 ml/kg.

Group II- Extract of *Actiniopteris dichotoma* was given at 250 mg/kg.

Group III - Extract of *Actiniopteris dichotoma* was given at 500 mg/kg.

Group IV – standard drug Paracetamol was given at 100 mg/kg.

The mice were positioned on the hot plate kept at a temperature of 55°C; the reaction time was recorded when animals licked their fore or hind paw, or jumped prior to and 0, 30, 60, 120, 180 and 240 minutes after oral administration of the samples.[8] The latency time for all group was recorded at 0, 30, 60, 90, 120 min.

Tail Immersion Method :

Albino mice of either sex were devided into four groups each of 6 animal (20-25gm).

Group I- control group treated with normal saline 5 ml/kg

Group ll- standard paracetamol was given at the dose of 100 mg/kg

Group lll- extract at the dose of 250 mg/kg was given

Group lV- extract at the dose of 500 mg/kg was given

The animal was kept in vertical position to long the fail which was up to 5 cm. into the pot of hot water maintained at 55 ± 0.5° c. The timing in second to withdrawal the fail out of water was taken as the reaction time (Ta). The reading was taken after 0, 30, 60, 90, 120 min. of administration of test drug. The cut off time i.e. time of no response was put at 30 second, while Tb was consider the reaction time for control group.[8]

STATISTICAL ANALYSIS

The values were represented as mean ± SEM. and the data obtained from this study was subjected to one-way analysis of variance (ANOVA) followed dunnet's test. The values of ***p<0.001, *p<0.01, **p<0.05 were considered to indicate the significant levels.

RESULTS AND OERVATION

Acute Oral Toxicity

The acute toxicity study showed that animals fed by oral gavages tolerated the limit dose of 5000 mg/kg body weight of methanolic extract of ***Actiniopteris dichotoma***. There were no visible signs of acute toxicity during the 14 days of observation. Absence of death at all doses up to 5000 mg mg/kg showed that the LD50 of the extract is greater than 5000 mg extract/kg body weight. There was a significant increased in weight gain of rats after 14 days of extract treatment at 5000 mg/kg as compared with control. The eating, drinking habit and behaviour of all the animals used were normal. The results obtained on the average water and food intake and weekly weight gain are presented.

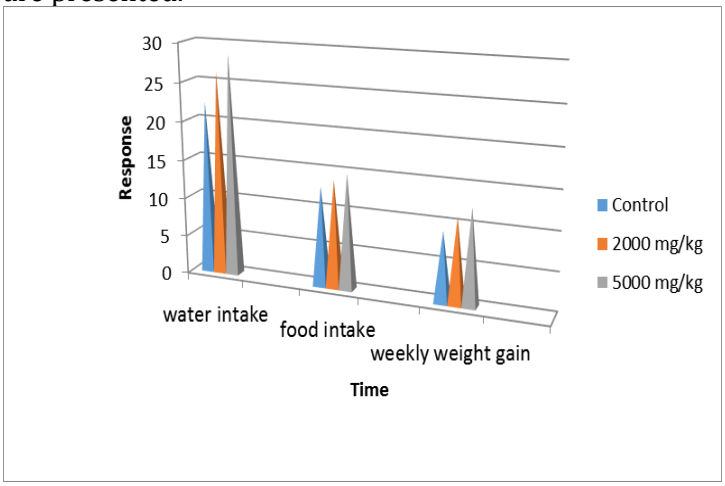

Fig. 1- acute oral toxicity study

ANTI-INFLAMMATORY ACTIVITY-

Carrageenan induced paw edema model-

The result of anti-inflammatory activity of methanolic extract of ***Actiniopteris dichotoma bedd*** at two different doses (250 mg/kg, 500mg/kg) on carrageenan induced paw odema is shown in table.

The methanolic extract and indomethacin showed inhibition at early and late phase. At the 250 mg/kg dose of methanolic extract of *Actinopteris dichotoma* showed inhibition ($P < 0.05$) and the maximum inhibition of methanolic extract at 500 mg/kg at the time of 180 min and 240 min ($P < 0.01$). The standard indomethacin also showed maximum inhibition at 120 min, 180 min, 240 min ($P < 0.01$). In this model methanolic extract of *Actinopteris dichotoma* bedd at 500 mg/kg showed significant inhibition of odema formation when compare to vehicle control group.

Table 1- Effect of methanolic extract of *Actiniopteris dichotoma bedd* on Carrageenan Induced Hind Paw Edema of Rat.

Drug	Dose	0 min	60 min	120 min	180 min	240 min
Control Negative	Normal saline 5ml/kg	0.34±0.04	0.36 ± 0.03	0.41±0.02	0.39±0.01	0.38±0.01
Indomethacin	5 mg/kg	0.29±0.02	0.17 ± 0.02	0.16±0.07	0.14±0.01	0.12±0.01
Actiniopteris dichotoma	250 mg/kg	0.31±0.04	0.26 ± 0.03	0.22±0.04	0.20±0.05 *	0.17±0.04 *
Actiniopteris dichotoma	500 mg/kg	0.29±0.02	0.23 ± 0.04	0.19±0.07	0.17±0.02**	0.13±0.01**

Values are reported as mean ± S.E.M. for group of six animals, data was analised by ANOVA followed by Dunnett's test. Significant value from control P < 0.05 * P < 0.01**

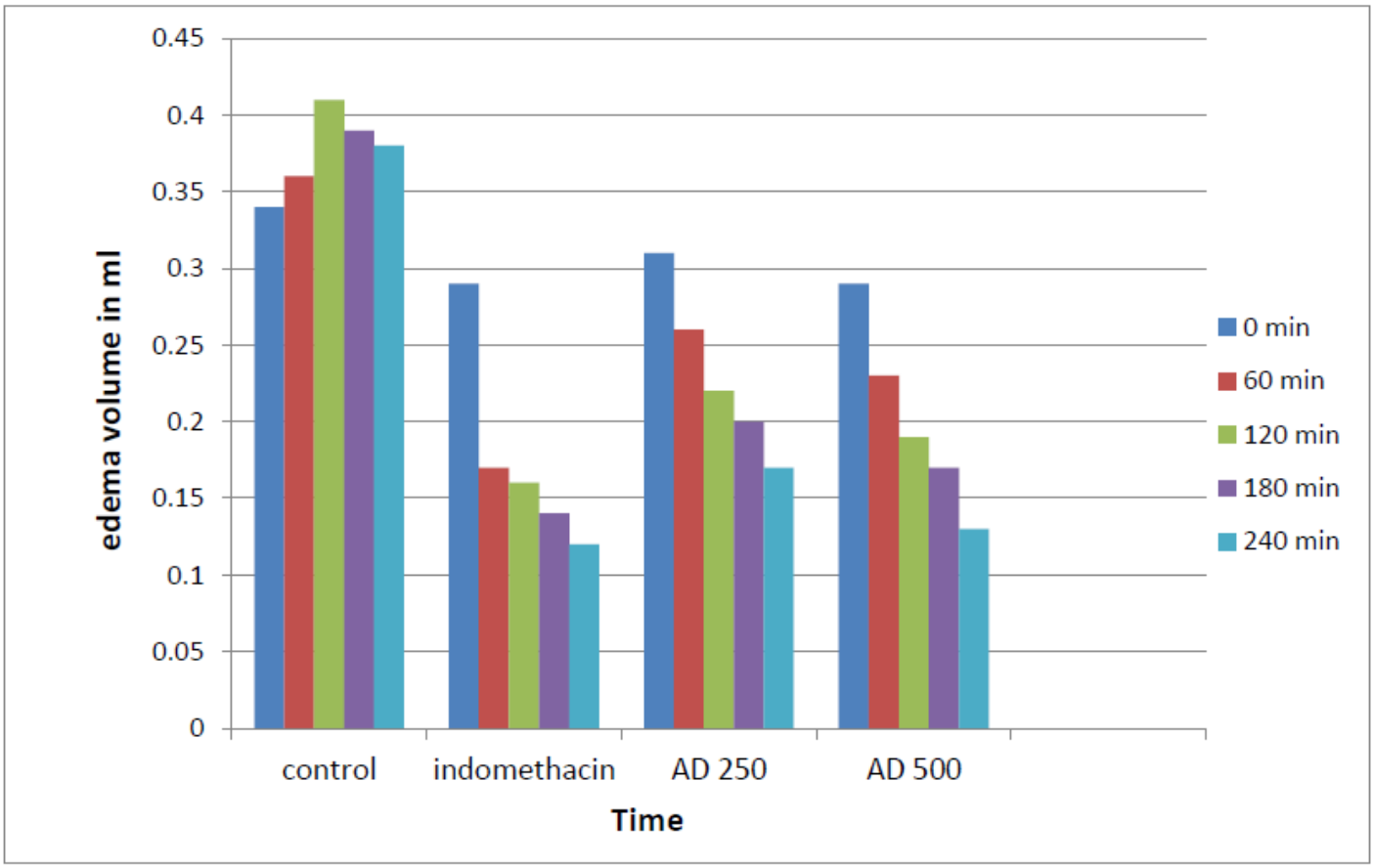

Figure 2-Effect of Methanolic Extract of *Actiniopteris dichotoma bedd* on Carrageenan Induced Hind Paw Edema of Rat

Histamine induced paw edema model-

The result of anti-inflammatory activity of methanolic extract of ***Actiniopteris dichotoma bedd*** at two different doses (250 mg/kg, 500mg/kg) on carrageenan induced paw odema is shown in table.

Methanolic extract and indomethacin showed significant inhibition against histamine induced odema. At the 250 mg/kg dose of methanolic extract of *Actinopteris dichotoma* showed inhibition (P < 0.05) and the maximum inhibition of methanolic extract at 500 mg/kg at the time of 180 min and 240 min (P < 0.01). The standard indomethacin also showed maximum inhibition at 120 min, 180 min, 240 min (P < 0.01). In this model methanolic extract of *Actinopteris dichotoma* bedd at 500 mg/kg showed significant inhibition of odema formation when compare to vehicle control group.

Table 2- Effect of methanolic extract of *Actiniopteris dichotoma bedd* in Histamine induced paw edema.

Drug	Dose	0 min	60 min	120 min	180 min	240 min
Control Negative	Normal saline 5ml/kg	0.35±0.05	0.37 ± 0.04	0.39±0.04	0.42±0.01	0.39±0.01
Indomethacin	5 mg/kg	0.32±0.02	0.19 ± 0.04	0.18±0.03	0.16±0.01	0.12±0.02
Actiniopteris dichotoma	250 mg/kg	0.34±0.02	0.27± 0.03	0.22±0.05	0.21±0.02	0.16±0.02*
Actiniopteris dichotoma	500 mg/kg	0.34±0.01	0.24± 0.01	0.18±0.02**	0.16±0.02**	0.14±0.02**

Values are reported as mean ± S.E.M. for group of six animals, data was analised by ANOVA followed by Dunnett's test. Significant value from control P < 0.05 * P < 0.01**

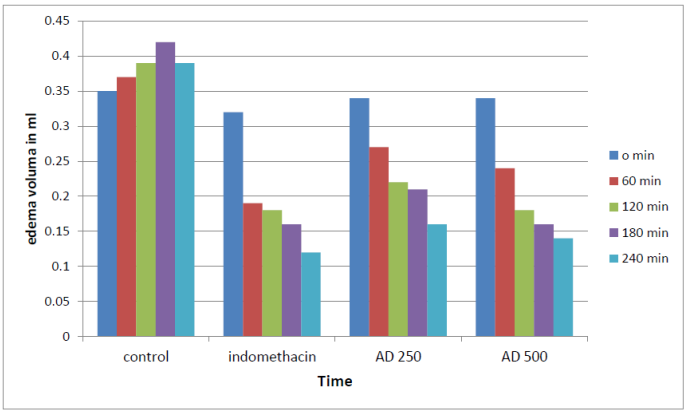

Figure 3-Effect of Methanolic Extract of *Actiniopteris dichotoma bedd* on Histamine Induced Hind Paw Edema of Rat.

ANALGASIC ACTIVITY –

Hot plate method-

The results of the hot plat test revealed that the latency time was significantly (P<0.05) increased from 18.44% to 67.66% at the dose of 250 mg/kg at 60 min. and at the dose of 500 mg/kg at 30 min. The effect was dose dependent and the maximum effect was observed after 60 min at the dose of 250 mg/kg and 500 mg/kg observed extract . The most significant (P<0.01) increase in latency time noticed against 500mg/ kg of *Actinopteris dichotoma* was 67.66% whereas, the percent inhibition of the standard drug was 76.73% .

Table 3- Effect of methanolic extract of *Actiniopteris dichotoma bedd* in hot plat test

Drug	Dose	0 min	30 min	60 min	90 min	120 min
Control Negative	Normal saline 5ml/kg	9.25± 0.05	9.28 ± 0.04	9.15 ± 0.08	9.021± 0.03	9.016± 0.12
Standard Paracetamol	100 mg/kg	9.24 ± 0.04	24.24 ± 0.06**	24.58± .08***	24.95± .07***	24.78± 0.01***
Actiniopteris dichotoma	250 mg/kg	9.28 ± 0.45	11.89± 0.67	13.15± .45**	13.45± 0.48**	13.14± 0.19**
Actiniopteris dichotoma	500 mg/kg	9.20 ± 0.67	18.67± 0.21*	22.47± .21**	23.15± 0.21**	22.48± 0.16**

Values are reported as mean ± S.E.M. for group of six animals data was analised by ANOVA followed by Dunnett's test. Significant value from control P < 0.05*, P < 0.01**, P < 0.001***

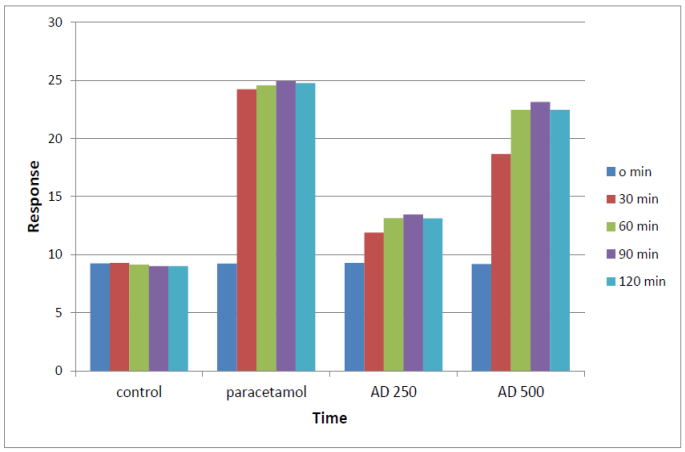

Figure 4-Effect of methanolic extract of *Actiniopteris dichotoma bedd* in hot plat test

Tail immersion method:

Analgesic effect the actiniopteris dichotoma was also significant P < 0.05 in tail immersion test and this was also dose dependant like hot plate test. The reaction time of all dose and standard drug was given in table. The maximum analgesic effect was taken at 60 min after the dose administration. The % inhibition of pain was 18.44% to 67.66% at 250 and 500 mg/kg of methanolic extract of *Actinopteris dichotoma* respectively. Paracetamol standard drug is showed maximum activity 77.74%.

Table 4- Effect of methanolic extract of *Actiniopteris dichotoma bedd* in Tail immersion test

Drug	Dose	0 min	30 min	60 min	90 min	120 min
Control Negative	Normal saline5ml/kg	3.25± 0.02	3.26 ± 0.05	3.37± 0.05	3.29 ± 0.9	3.26± 0.14
Standard Paracetamol	100 mg/kg	3.22 ±0.02	5.67±0.04***	5.98±0.06***	5.84±0.05***	5.79±0.00***
Actiniopteris dichotoma	250 mg/kg	3.25 ± 0.25	3.68± 0.24*	3.98± 0.18**	3.98± 0.45**	3.89± 0.97**
Actiniopteris dichotoma	500 mg/kg	3.27 ± 0.28	4.95 ± 0.24*	5.78± 0.62**	5.45± 0.71**	5.40± 0.28**

Values are reported as mean ± S.E.M. for group of six animals data was analised by anova followed by Dunnett's test. Significant value from control P < 0.05*, P < 0.01**, P < 0.001***

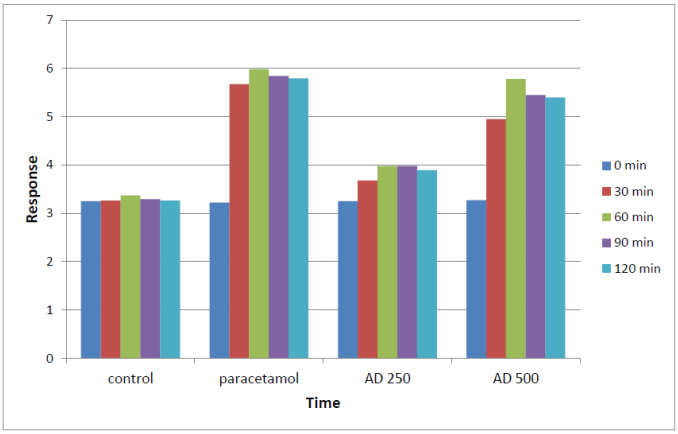

Figure 5-Effect of methanolic extract of *Actiniopteris dichotoma bedd* in Tail immersion test

DISCUSSION

Acute Toxicity Study

In the acute toxicity study showed that animals were tolerated the limit dose of 5000mg/kg body weight of methanolic extract of *Actiniopteris dichotoma bedd*, there was no sign of acute toxicity during the 14 days observation. At the 2000mg/kg and 5000mg/kg slightly changes were recorded as compare to control maximum changes were obtained to average water intake, average food intake and average weight gain at the 5000mg/kg. the significant difference ($P<0.05$) as compare to control were obtained of different parameters.

Anti-Inflammatory Activity

The anti-inflammatory activity of methanolic extract of *Actiniopteris dichotoma bedd* at two different doses (250 mg/kg, 500mg/kg) on carrageenan induced paw odema. Carrageenan induced odema falls in the category of acute inflammation which is due to the release of inflammatory mediators at site of injury which cause pain and fever. The early phase of the carrageenan model mediated by serotonin, histamine and prostaglandin releasing. The late phase of inflammation sustained by PGs releasing and mediated by bredykinin and leukotrins. Histamine induced odema in earlier stage of vascular reaction in the chemically induced inflammation in which swelling occurs due to histamine. Histamine is released from the mast cell degranulation by no. of inflammatory mediators such as substance P, interleukin 1. Methanolic extract and indomethacin showed significant inhibition against histamine induced odema.

Analgesic Activity

The study indicated that *Actinopteris dichotoma* bedd has peripheral and central analgesic properties. In analgesic activity test of the methanolic extract of *Actiniopteris dichotoma bedd*, experimental animal models included the hot plate and tail immersion tests were used in this study. The *Actiniopteris dichotoma bedd* clearly demonstrated analgesic activity in both experimental animal models used in this study. Therefore, these result could be implied that the methanolic extract of *Actinopteris dichotoma* bedd had analgesic mechanisms in central nervous system centrally mediated.

REFERENCES:

1) Dubey N.K. *et al.,* Global promotion of herbal medicine: India's opportunity. CURRENT SCIENCE, VOL. 86, NO. 1, 10 JANUARY 2004, page no. 37-41.
2) Raymond francis, Inflammation: A common denominator of disease, Beyond health copyright 2007.
3) Patel B. Nilesh "physiology of pain" ; Internation association for the study of pain 2010. Page no. 13-16.
4) OECD, "Guidelines for the testing of chemicals / section 4: Health effects test no. 423: Acute oral toxicity - Acute toxic class method," Organization for Economic Cooperation and Development, Paris, France, 2002.
5) Sindhu.A, Muraleedharn M.P Microbial Growth Inhibition By Aparajitha Dhooma Choornam. Ancient Science of Life, Vol : XXVI (3&4) January, February, March 2007.
6) UMA.G* Balasubramaniam.V and Jagathes kumar.S, " In-vivo screening of anti-inflammatory activity in methanolic extract of corbichonia decumbers using various animal models of Paw oedema. International journal of pharmacy and pharmaceutical science; vol 6, issue 1, 2014.
7) Kulkarni S.K. "Hand book of experimental pharmacology", vallabh prakashan. Page no. 125-126.
8) S.T. Sourabie et al. "Biological evaluation of anti-inflammatory and analgesic activities of Argemone Mexicana linn. (Papaveraceae) aqueous leaf extract"; Internation journal of pharma sciences and research (IJPSR). ISSN: 0975-9492. Vol 3 no. 9 sep 2012. Page no. 453.

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