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Research Article

A PRELIMINARY STUDY ON ANTI-INFLAMMATORY ACTIVITY & ANTIOXIDANT PROPERTY OF *LYGODIUM FLEXUOSUM*, A CLIMBING FERN.

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ABSTRACT

Fronds from the climber fern, *Lygodium flexuosum* (L) Sw. were investigated for its anti- inflammatory role in mice. The ethanolic and aqueous extracts at two doses of 250 mg/kg and 500mg/kg were assessed to determine the anti inflammatory potential in Carrageenan induced paw edema model using Indomethacin (10mg/kg) as a standard. The results indicated that the ethanolic extract produced significant (P<0.001) anti-inflammatory activity as compared to untreated control group. The antioxidant activity being vital in provoking anti-inflammatory activity was carried out by monitoring the ABTS radical and H_2O_2 radical scavenging in plant extracts alone. Ascorbic acid was used in both the *invitro* anti-oxidant assays. The parameters undertaken suggest the fern to possess both anti-inflammatory and free radical scavenging ability.

Keywords: *Lygodium flexuosum*, Anti-inflammatory, ABTS radical, H_2O_2.

INTRODUCTION

Oxidative stress is the outcome of generation of free radicals which is partly or wholly responsible for various ailments now-a-days. Formation of free radicals is mainly due to less antioxidant production which can be supplemented by external agents such as plant and plant products[1]. According to Javanmardi *et al.,* 2003, antioxidants play an important role in absorbing and neutralizing some free radicals, quenching singlet and triplet oxygen or directly decomposing peroxides[2]. A strong correlation exists between inflammation and tissue injury related to oxidative stress.

Inflammation is believed to be a defensive mechanism of the body to remove the injurious stimuli as well as initiate the healing process for the tissue[3].

Inflammation is a biphasic process, the early phase (1-2 hours) which is mainly mediated by mediators like histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue and the late phase which is sustained by prostaglandin release and mediated by bradykinin, leukotrienes [4].

Manifestation of inflammation involves, vasodilation which includes increased blood flow causing the redness (*rubor*) and increased heat (*calor*). Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which produces swelling (*tumor*). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia, *dolor*) [5].

Herbal drugs and natural products have been known to human being for many years and they have been used as a source of different therapy and treatment of many diseases [6]. According to Ismail, 2010 WHO recognized herbal medicine as an important factor in primary health care in India [7]. Considering many side effects caused by synthetic allopathic drugs, more urge to herbal drugs with equal effects have emerged [8]. For eg, long term use of NSAIDs may cause peptic ulcers as its side effects [9]. Hence, we have focused on *Lygodium flexuosum*, a climber which is a pteridophyte belonging to the family Schizaeaceae. It is commonly known as 'Bhutraj' or 'Maiden hair creeper' [10] which is an important medicinal plant. Fresh roots are used in external application for rheumatism, sprains, scabies, and eczema and cut wounds while leaves are used to treat boils [11, 12]. In a previous report of Jeetendra *et al* 2011 the whole plants has an ethnomedicinal value as it is used in the treatment of jaundice [13].

MATERIALS AND METHODS

Collection and authentication

The fern *Lygodium flexuosum* (LF) was collected during monsoon from Dapoli, Ratnagiri District of Maharashtra. The Herbarium was prepared and authenticated from Botanical Survey of India, Pune under the voucher no BSI/WC/TECH/2011/307 by Dr P.G. Diwakar.

Preparation of Plant extract:

The fronds were washed and shade dried in a dryer for 48 hrs and moisture content was determined. The dried fronds obtained were crushed and kept in the Soxhlet apparatus for 24 hrs for obtaining ethanolic and aqueous extract by their respective solvents. The aqueous as well as ethanolic extract obtained was concentrated in rotary evaporator under vacuum and their percent yield was determined.

Chemicals and Drugs

Carrageenan, were obtained from Sigma-Aldrich. Tween 80, Carboxy methyl cellulose (CMC) from Fisher Scientific, Mumbai The standard Indomethacin was obtained from Sigma-Aldrich. All the chemicals and drugs used were of analytical grade.

Animals

Swiss albino mice (22-25gms) were used in the study. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2^0 C having 50±5% relative humidity with 12-hour light and dark cycle. Animals were fed a standard laboratory diet with water *ad libitum*. The use and care of the animals in the experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC) on 30/3/2011. The animal experimentation was carried out following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA)

Acute toxicity study

The acute toxicity study was performed in female Swiss Albino mice (18-20 g) according to the OECD guidelines 423. The animals were dosed once with 2 gm/kg of the extracts respectively and observed for 14 days [14]. Lack of morbidity and mortality at the end of 14 days suggested that 2gm/kg of the extracts to be safe for further experimentation, of which 500mg/kg and 250 mg/kg were selected for the present study.

Anti-inflammatory Activity

Carrageenan induced mice paw edema method

Anti-inflammatory activity was assessed by the method described by M Al-Amin *et al* 2011[15]

In brief Swiss albino mice weighing 22-25 gm were divided into 6 groups (n=6).

Group-I received 0.5% Carboxy methyl cellulose (CMC) suspension (control),

Group-II received Indomethacin (reference drug 10 mg/kg, p.o),

Group-III, IV received aqueous extracts (250 mg/kg and 500mg/kg, p.o) and

Group- V and VI received ethanolic extracts (250 mg/kg and 500 mg/kg, p.o), respectively.

Subsequently, 1 hour after dosing, 0.05 ml of 1% suspension of Carrageenan was injected into the sub-planter region of left hind paw to induce edema. The paw edema was measured with a Vernier Caliper [16] initially and then Carrageenan was injected, after which the paw volume was measured at 0, 1, 2, 4 and 6 hours respectively. The difference between the initial and subsequent values gave the actual edema which was compared with the control animals. The percent inhibition of inflammation was calculated using the following formula

$$\% \text{ inhibition} = C\text{-}T/C \times 100$$

Where, 'C' represents mean edema in control and 'T' represents mean edema in group treated with standard drug and test drug.

ABTS decolorization assay

The ABTS radical scavenging activity was analyzed according to the method of Re and coworker [17]. ABTS was dissolved in distilled water to a concentration of 7mmol/L. ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution with 2.45mmol/L of Potassium persulfate[18] and the mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. The percent scavenging activity of the plant extract was determined by carrying out the percent inhibition which was calculated by the following formula and results were compared with ascorbic acid as standard.

$$\% \text{ inhibition} = \frac{\text{Absorbance control - Absorbance test}}{\text{Absorbance control}} \times 100$$

The concentration equivalent to ascorbic acid was calculated by plotting the values of the test extracts on standard curve of ascorbic acid [19].

Hydrogen peroxide (H_2O_2) scavenging assay

The ability of the *Lygodium flexuosum* to scavenge hydrogen peroxide was determined according to the method of Ruch *et al* [20]. Plant extract (2ml) prepared by distilled water at various concentration were mixed with 0.3ml of 4mm H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2.

Statistical analysis

The results were analyzed for statistical significance by one way ANOVA and were expressed as Mean + SEM by using Graphpad prism 5 version (Dunnett's test).Values representing $P<0.001$ were considered as statistically significant.

RESULTS

Effect of *Lygodium flexuosum* in Carrageenan induced paw edema

The aqueous and ethanolic extracts of LF at the two doses of 250 mg/kg and 500 mg/kg showed a significant reduction in the paw oedema volume at an interval of 6 hrs after Carrageenan treatment. The ethanolic fraction showed an improved activity as compared to the aqueous extract with a maximal effect at 500 mg/kg which was greater than the effect produced by Indomethacin after 60 minutes Table1.

Table 1: Effect of *Lygodium flexuosum* on paw edema volume

Group	Treatment	Dose/kg p.o	Initial paw vol.	0 hr	1hr	2hr	4hr	6hr
isease control	Saline	0.5ml	2.11±0.02	3.14±0.06	3.28±0.051	3.63±0.02	3.81±0.04	3.62±0.03
standard drug	Indomethacin	10mg/kg	2.08±0.02^{ns}	3.02±0.05	3.10±0.027***	2.96±0.02***	2.37±0.03***	2.28±0.02***
test drug 1	LF aq	250mg/kg	2.14±0.03^{ns}	3.09±0.09	3.13±0.028***	2.67±0.03***	2.31±0.04***	2.23±0.03***
test drug 2	LF aq	500mg/kg	2.09±0.03^{ns}	3.06±0.05	3.10±0.026***	2.64±0.03***	2.48±0.03***	2.20±0.04***
test drug 3	LF eth	250mg/kg	2.12±0.03^{ns}	3.03±0.12	3.11±0.025***	2.49±0.03***	2.27±0.03***	2.19±0.03***
test drug 4	LF eth	500mg/kg	2.14±0.03^{ns}	3.10±0.08	2.99±0.031***	2.47±0.02***	2.20±0.02***	2.15±0.04***

Data: Mean ± SEM of n=6 $^{***}P<0.001$, and 'ns' non significant when compared with disease control.

Effect of *Lygodium flexuosum* on free radical scavenging by ABTS method

The aqueous extract at 250µg/ml showed 66.35% inhibition whereas the ethanolic extract at 250µg/ml showed 72.62% inhibition. The concentration of the aqueous extract at 250µg/ml was equivalent to 37.33µg/ml of ascorbic acid whereas the concentration of the ethanolic extract at 250µg/ml was equivalent to 41.17 µg/ml of ascorbic acid when plotted on standard curve of ascorbic acid Table 2. Five concentrations (5 to 50µg/ml) of standard ascorbic acid were prepared of which 50 µg/ml showed 100 % inhibition of ABTS radical formation.

Effect of *Lygodium flexuosum* on Hydrogen Peroxide radical scavenging activity

The ethanolic extract at 250µg/ml showed 40.57% inhibition whereas the aqueous extract at 250µg/ml showed 35.93% inhibition of hydrogen peroxide. Ascorbic acid was used as standard which at a highest concentration of 25µg/ml showed 50% inhibition of hydrogen peroxide. Figure 1.

Table 2: ABTS radical scavenging activity of *Lygodium flexuosum*

Groups	Concentration (µg/ml)	% Inhibition	Concentration equivalent to ascorbic acid
LF Aq	10	11.04±0.54	3.50
	50	16.89±0.22	7.08
	100	21.93±0.33	10.17
	150	43.73±0.36	23.50
	200	48.77±0.47	26.58
	250	66.35±0.33	37.33
LF Eth	10	15.12±0.23	6.00
	50	18.39±0.21	8.00
	100	27.25±0.31	13.42
	150	33.79±0.23	17.42
	200	57.90±0.33	32.17
	250	72.62±0.42	41.17

Data: Mean ± SEM (in triplicate) (correlation coefficient R^2=0.976 of standard curve of ascorbic acid)

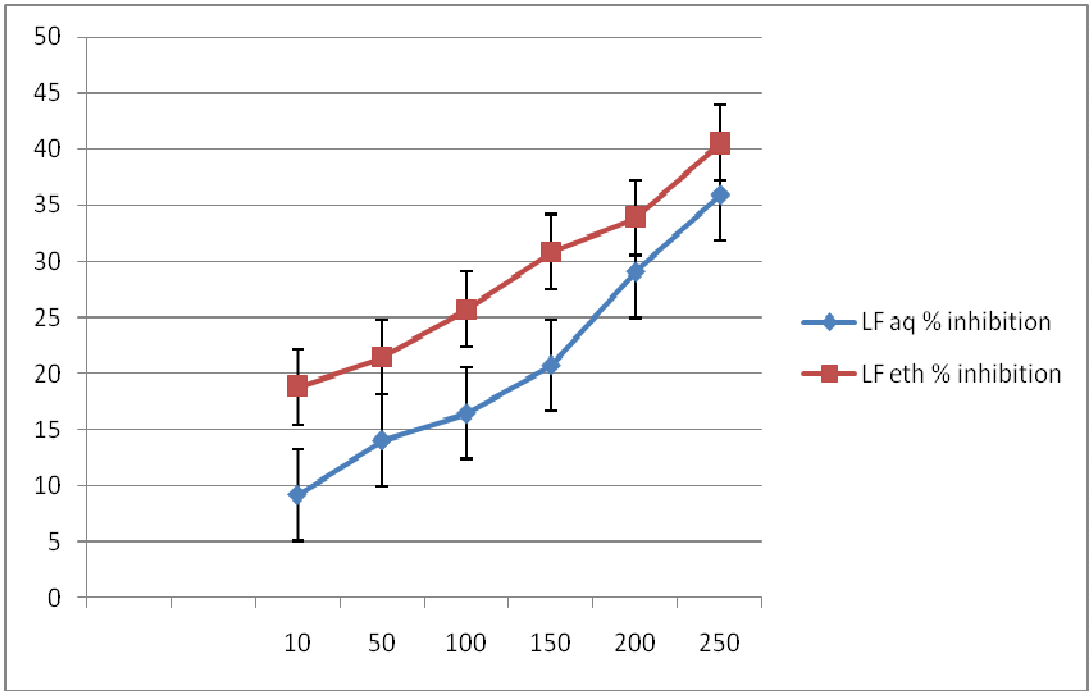

Fig. 1: H_2O_2 radical scavenging activity of *Lygodium flexuosum*

DISCUSSION

The most important mediators that provoke inflammatory processes are reactive oxygen species and consequently their annihilation by antioxidants and radical scavenger can alleviate inflammation. Plants having antioxidant components are thus often found to exhibit better anti-inflammatory activity [21]. The most extensively employed test to evaluate anti-inflammatory agents is the ability of a compound to inhibit paw edema induced by injection of a phlogistic agent like Carrageenan. Presently, a gradual increase in the paw volume were observed in the control animals while a significant reduction in the paw edema was observed in the test extract treated groups when compared with the control group. The activity of the two test extracts of LF at two doses were similar to that exhibited by the group treated with Indomethacin 10mg/kg. The highest percentage inhibition was found in the dose of 500mg/kg of the ethanolic extract with the mean percentage inhibition of 30.42% whereas the ethanolic extract treated mice at 250mg/kg dose showed paw volume reduction of 29.03%. Similarly, the aqueous extract at 500mg/kg and 250mg/kg showed approximately 29.58% and 28.75% reduction of paw edema respectively which might be attributed to the antioxidant potential of the fern.

Thus, the ABTS assay was carried out for assessing the free radical scavenging antioxidant activity of extracts. The method is based on the ability of antioxidant molecules to quench the ABTS radical cation ($ABTS^{+}$) [22] and excessive presence of antioxidant potential leads to rapid discolouration of the greenish blue complex. Significant ABTS free radical scavenging activity was evident in both ethanolic as well as aqueous extracts. To further potentiate the antioxidant prospective the hydrogen peroxide scavenging assay was performed. According to Kerr Me *et al*, 1991 [23, 24] hydrogen peroxide is a non-radical form of reactive oxygen species that is formed in living organisms by superoxide dismutase. Moreover, it can cross biological membranes and generates hydroxyl radicals which are toxic to cells. Plant products by various enzymatic and non-enzymatic mechanism of action can scavenge these hydroxyl radicals and protect the cells and biomolecules against reactive oxygen species.[25]

The present study demonstrated that the ethanolic and aqueous extracts of *Lygodium flexuosum* showed promising antioxidant and radical scavenging activities at various concentrations. From the observations it can be concluded that the fronds of *Lygodium flexuosum* are the good sources of natural antioxidants and might be useful in treating the diseases associated inflammation.

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