# DIFFERENTIAL INHIBITORY POTENCIES OF ALCOHOLIC EXTRACT OF DIFFERENT PARTS OF *DRYOPTERIS CHRYSOCOMA* ON INFLAMMATION IN MICE AND RATS

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**ABSTRACT**

Phytomedicinal therapy for inflammation is not new and it is highly effective for the treatment of various inflammatory disorders. The inflammation is one of the initial parameter for most of the disorders occurring in the body. The anti-inflammatory potential can be determined by using various techniques. *Dryopteris chrysocoma* is a male fern commonly found in damp and moist areas of Pakistan. The study was conducted on mice and rats by inducing inflammation with subcutaneous administration of formalin and Carrageenan in hind paw. The results were compared with standard drug Aspirin administered orally in the dose of 300 mg/kg and a decrease in hind paw volume was observed. The intensity of edema was observed in mice after formalin injection and the time of disappearance of edema was observed. In rats the inhibition of inflammation by root, leaves and stem extract was 51.19%, 41.66% and 30.95% respectively after administration of formalin. Similar inhibition of inflammation produced by root, leaves and stem extracts *i.e.* 57%, 42% and 35% respectively in Carrageenan treated rats. Root extract showed the highly significant results at $p \leq 0.05$. The comparative study explored the root extract has more potent anti-inflammatory activity than leaves extract. The stem extract has less potent anti-inflammatory action.

**Keywords**: Male fern, *Dryopteris chrysocoma,* formalin, Carrageenan; Inflammation.

## INTRODUCTION

*Dryopteris chrysocoma* is a fern, an evergreen plant, grows to a height of 1m and produces seeds in July to September. It grows abundantly in the temperate regions of Asia, British Isles and many areas of Europe (Clapham *et al*., 1990). The reported chemical constituents of it are albaspidin, filmaron, oleoresin, flavaspidic acid and filicic acid (Bhattacharjee, 2004). Phloroglucinol derivatives of crude filicin are present in the rhizomes of *D. chrysocoma*. Phloroglucinol and the reported derivatives are albaspidin, flavaspidic and filixic acids (Asolkar *et al*., 1992). The male fern root is commonly used as anthelmintic. Usually fresh plant parts are used because with the passage of time it loses its efficacy. The major active chemical constituents responsible for the activity are filmarone and aspidinol. These compounds are effective in killing tap worms (Weiss, 2001).

## MATERIALS AND METHODS

### *Plant material*

In the month of May 2004 fresh male fern i.e., *D. chrysocoma* was collected from the Karachi University than after washing and drying it was cut in to small pieces and then soaked in methanol.

The voucher specimen (No. 001006-09 of *Dryopteris chrysocoma* (root), No. 001006-08 of *Dryopteris chrysocoma* (leaves)), No. 001006-07 of *Dryopteris chrysocoma* (stem) were deposited in herbarium of Department of Phamacognosy, University of Karachi.

### *Preparation of plant extracts*

The fresh plant material (leaves 1kg, stem 1kg and roots 2kg) was cut in to small pieces separately and then methanol (2 lit for leaves, 1.5 lit for stem and 3 lit for roots) was added and the material was kept at room temperature for 15days (repeated this procedure three time for complete extraction). After that methanol extract was separated from plant material via filtration (Whatman No.3 filter paper) and then solvent was evaporated under reduced pressure on a rotary evaporator (Buchi rotavapor R-200, Switzerland). A semi-solid material was obtained weighing 40g (root), 20g (leaves) and 15g (stem). A part from these crude extracts was used during experiments.

### *Experimental Animals*

The study was carried out on Wistar albino rats (150-200 g) of either sex. The rats were bred in colony in the

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Animal House of the faculty of Pharmacy. They were fed with a standard pellet diet and water *ad libitum*. Before their use in experiments the mice were kept in standard environmental conditions, (temperature 25-28°C and 12 h light/dark cycle). Five animals in each group were used in all sets of experiments. A proper permission was obtained from Ethical Committee of Faculty of Pharmacy, University of Karachi, Karachi, Pakistan, and after the use animals were properly disposed off, according to standard procedure.

***Anti inflammatory Activity***

**Formalin Test in Mice for screening purpose**

Modified method of Hunskaar and Hole (1987) and Rathi *et al.* (2003) was used in observing the anti-inflammatory activity where formalin is injected in animals to produced edema. The test was performed on mice and eight groups of five animals each were used in the test. Formalin (2%) 20 µL was administered in the ventral surface of the right hind paw. The observations were made for the 5 hours. Standard group of the mice received Aspirin in the dose of 300 mg/kg while the crude drug was administered in the dose of 300 and 500 mg/kg before 30 minutes of the subcutaneous administration of formalin in hind paw. The control group received only vehicle before 30 minutes of the subcutaneous administration of the formalin in the right hind paw. The total time spent in the production and disappearance of edema was observed and compared with standard drug and control. It was found significant (table 1).

***Formalin injected rat paw edema measurement by vernier caliper method***

Rats were divide in to eight groups (n=5). The rats received a subcutaneous injection of 100 µL of formalin solution in to the plantar surface of the right hind paw with a 26-gauge needle after 30 minutes of the oral administration of drugs. Standard group of the rats received Aspirin in the dose of 300 mg/kg. Treated groups received crude drug was administered in the dose of 300 and 500 mg/kg before 30 minutes of the subcutaneous administration of formalin in hind paw. The control group received only 0.5 ml vehicle before 30 minutes of the subcutaneous administration of the formalin in the right hind paw. The rats were then placed in an individual cage. The baseline diameters of the hind paws were measured before the formalin injection using a Vernier caliper; at the metatarsal level. Those of the hind paws that developed edema were determined at 1, 2, 3 and 4 hr after the injection by measuring the dorsal plantar foot thickness at the metatarsal. The time spent in the production and disappearance of edema was observed and compared with standard drug and control. It was found significant (table 2) (Brown *et al*., 1968; Lee and Crosby, 1999).

**Table 1**: Assessment of intensity of edema in mice after formalin injection

| Treatment | Dose | 0hour | 1hour | 2hour | 3hour | 4hour | 5hour |
|---|---|---|---|---|---|---|---|
| Control | 0.5ml saline | - | ++ | +++ | +++ | +++ | +++ |
| *D. chrysocoma* (root) | 300mg/kg | - | + | ++ | ++ | + | + |
| | 500mg/kg | - | + | ++ | ++ | + | - |
| *D. chrysocoma* (leaves) | 300mg/kg | - | + | +++ | ++ | + | + |
| | 500mg/kg | - | + | ++ | ++ | + | + |
| *D. chrysocoma* (stem) | 300mg/kg | - | ++ | +++ | ++ | ++ | + |
| | 500mg/kg | - | + | +++ | ++ | ++ | + |
| Aspirin | 300mg/kg | - | + | ++ | + | + | + |

Intensity of edema is shown by positive and negative signs.+=Slight edema,++=moderate edema,+++=highly inflamed, -= no edema

**Table 2**: Assessment of anti-inflammatory activity by Formalin test in rats

| Treatment Groups | Dose | Diameter (cm) of hind paw | | | | |
|---|---|---|---|---|---|---|
| | | 0hour | 1hour | 2hour | 3 hour | 4 hour |
| Control | 0.5 ml | 0.31±0.02 | 0.91±0.05 | 0.92±0.03 | 0.92±0.03 | 0.84±0.03 |
| *D. chrysocoma* (root ) | 300 mg/kg | 0.33±0.04 | 0.56±0.02* | 0.56±0.02* | 0.55±0.05* | 0.44±0.04* |
| | 500 mg/kg | 0.32±0.10 | 0.55±0.04* | 0.54±0.02* | 0.54±0.03* | 0.41±0.03* |
| *D. chrysocoma* (leaves) | 300 mg/kg | 0.31±0.03 | 0.70±0.03 | 0.71±0.04 | 0.72±0.03 | 0.59±0.04* |
| | 500 mg/kg | 0.33±0.04 | 0.68±0.06* | 0.69±0.03* | 0.59±0.05* | 0.49±0.03* |
| *D. chrysocoma* (stem) | 300 mg/kg | 0.33±0.03 | 0.71±0.03 | 0.71±0.05 | 0.73±0.04 | 0.61±0.03 |
| | 500 mg/kg | 0.32±0.04 | 0.71±0.03 | 0.69±0.04* | 0.68±0.04* | 0.58±0.04* |
| Aspirin | 300 mg/kg | 0.31±0.02 | 0.62±0.04* | 0.63±0.03* | 0.63±0.03* | 0.52±0.02* |

Values represent the Mean ± SEM. N = 5; The results are expressed at $P \leq 0.05$. Statistically significant from control. * Significant

***Carrageenan injected rat paw edema measurement by plethysmometer***

The Wistar rats were divided into eight groups of five animals each. Group 1 served as positive control and received 0.5 ml vehicle. Group 2-7 received methanol extract, orally at a dose of 300 and500 mg/kg respectively. Group 8 received standard drug Aspirin, orally at a dose of 300 mg/kg. A mark was made on both the hind paws just beyond the tibiotarsal junction, so that every time the paw is dipped in the mercury column up to the marked level to ensure constant paw volume. After 1 hr of administration of the test and standard samples, 0.1 ml of 1% Carrageenan suspension (in normal saline) was injected into dorsal region of sub plantar surface of hind paw of rat subcutaneously with the help of 26 Gauge needle. The initial paw volume of each rat was recorded before drug administration. The paw volumes were measured at 0, 1, 2, 3 and 4 hrs using Plethysmometer. Any change in paw volume of rats was obtained by subtracting initial paw volume from the paw volumes at different time intervals. The average value of edema was calculated by taking the average of each group at different hours. Percentage inhibition of edema was calculated for each group with respect to its control group (table 3).

$$\text{Percentage (\%) inhibition} = \frac{(A - B)}{A} x100$$

Where A is the mean increase paw volume in rats treated with control and B is the mean increase in paw volume in rats treated with test drug (Turner, 1971; Handa *et al*., 1992).

## STATISTICAL ANALYSIS

The experimental data were calculated as mean ± SEM., evaluated by t-Test. Values of $P \leq 0.05$ were considered statistically significant.

## RESULTS

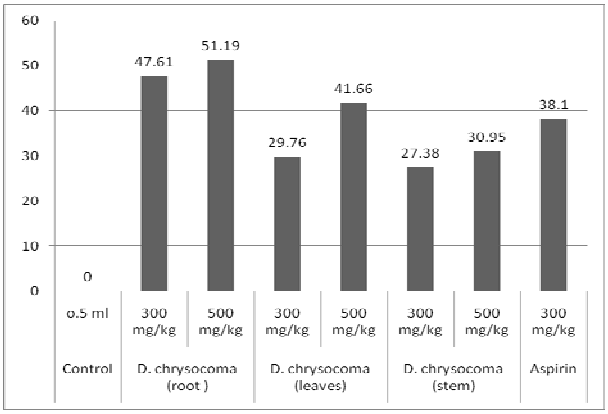
Anti-inflammatory potential of three separate parts of *D. chrysocoma* were determined by formalin tests and carrageenan induced inflammation in rats. The results of both tests showed strong anti-inflammatory potential of the crude extract (tables 1-3).

The edema induced after formalin injection was observed for five hours and it was found that slight edema was induced at 1 hour of the formalin injection and it was disappeared at the 5$^{th}$ hour of the formalin injection in the group of mice that received root extract of *D. chrysocoma*. The edema was also of low potency in the group of mice that received leaf and stem extract (table 1).

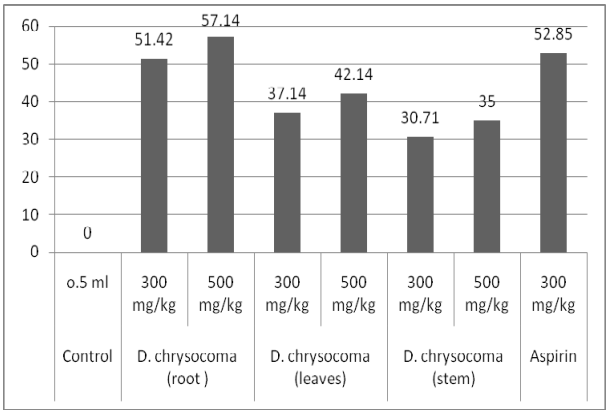
**Table 3**: Assessment of anti-inflammatory activity by Carrageenan induced inflammation in rats

| Treatment Groups | Dose | Edema volume ml (mean±SEM) of hind paw | | | | |
|---|---|---|---|---|---|---|
| | | 0 hr | 1 hour | 2 hour | 3 hour | 4 hour |
| Control | 0.5 ml | 0.56±0.05 | 0.65±0.06 | 0.81±0.02 | 0.96±0.08 | 1.4±0.03 |
| *D. chrysocoma* (root ) | 300 mg/kg | 0.51±0.04 | 0.58±0.01 | 0.65±0.02* | 0.71±0.04* | 0.68±0.08* |
| | 500 mg/kg | 0.53±0.03 | 0.56±0.03 | 0.60±0.01* | 0.59±0.02* | 0.60±0.02* |
| *D. chrysocoma* (leaves) | 300 mg/kg | 0.51±0.09 | 0.69±0.03 | 0.73±0.03 | 0.81±0.04 | 0.88±0.05* |
| | 500 mg/kg | 0.52±0.03 | 0.67±0.04 | 0.78±0.03 | 0.79±0.04 | 0.81±0.03* |
| *D. chrysocoma* (stem) | 300 mg/kg | 0.55±0.08 | 0.70±0.05 | 0.87±0.01 | 0.89±0.03 | 0.97±0.04* |
| | 500 mg/kg | 0.51±0.03 | 0.68±0.06 | 0.75±0.03 | 0.82±0.02* | 0.91±0.01* |
| Aspirin | 300 mg/kg | 0.50±0.02 | 0.59±0.07 | 0.61±0.07* | 0.66±0.02* | 0.66±0.03* |

Values represent the Mean ± SEM. N = 5; The results are expressed at $P \leq 0.05$. Statistically significant from control. * Significant




**Graph 1**: Percentage of inhibition of edema at 4hours after formalin injection in rats.




**Graph 2**: Percentage of inhibition of edema at 4hours after Carrageenan injection in rats.

The results of formalin activity in rats are expressed in table 2. Potent anti-inflammatory potential was found in root extract while leaf extract also possess strong anti-inflammatory activity (29.76%, 41.66%) but potency was less as compare to the roots extract (47.61%, 51.19%). The high dose of stem extract of the plant exhibited anti-inflammatory activity (27.38%, 30.95%). The results were compared with Aspirin at dose of 300 mg/kg. The anti-inflammatory potential of stem extract was less as compared to other parts of the plant but when compared with aspirin it was as high.

Anti-inflammatory potential was also determined by inducing inflammation with Carrageenan in the rat paw (table 3). Almost similar results were obtained as by the inflammation induced by formalin in mice and rats. The anti-inflammatory potential was observed at 1hr, 2hr, 3hr and 4hr of the subcutaneous administration of Carrageenan. Root extract produced highest anti-inflammatory potential (51.42% and 57.14%). The inhibition in edema that was an indicator of inhibition of inflammation was observed less after the administration of leaf (37.14% and 42.14%) and the stem extract (30.71% and 35%) and it was less as compare to the aspirin (52.85%) that was used as standard drug.

## DISCUSSION

Inflammation is one of the major symptoms of different diseases and disorders occur in human/animals. It is usually treated with different drugs of natural or synthetic origin but the utilization of plants to inhibit inflammation is still in common practice and the researches are trying to find out new sources. Therefore, in this regard Male fern is investigated and its anti-inflammatory action was determined by three different methods and each time same results were obtained with slight difference.

This plant is considered as a toxic plant because it is reported that in high doses it causes toxicity. Commonly it is utilized as an anthelmintic remedy (Duke *et al*., 1929). No anti-inflammatory research work was carried out prior to this report. The anti-inflammatory potential of *D. chrysocoma* was determined by performing experiments on mice and rats. The tests were performed on root, leaf and stem. The results obtained indicated the ability of inhibition of paw edema in different parts of plant and that is totally dependent on the occurrence of chemical constituents in that part. The studies suggest that the root extract has highest anti-inflammatory potential as compared to the leaf and stem extract. The lowest anti-inflammatory ability was observed in stem extract of the plant but it was also comparable with Aspirin (used as standard drug). Marc (2008) determined the decoction of *D. filix mas* is effective in gout as well as in rheumatism (Marc *et al.,* 2008). Saito in 1996 prepared tablets for allergy and inflammation. These tablets contain different ingredients including (-)-epigalocatechin gallate-4'-O- α -D-glucopyranoside, mannitol, potato starch and magnesium stearate (Saito *et al*., 1996). One of the scientists Fraunfelder determined the use of *filix mas* and also utilized *D. filix mas* for the treatment of ophthalmic disorders (Fraunfelder 2004). The present studies suggest that *D. chrysocoma* crude extract has high potency to reduce edema induced by formalin in the right hind paw of the mice. The root extract produced the maximum effect and the potency reduced as the leaf and then the methanolic extract of stem of *D. chrysocoma*. The inhibition of edema was comparable with Aspirin and control. During studies on animal models it was also observed that high dose of root extract produced death in animals. As is induced it causes severe depression and paralysis too. But at lower doses it exhibit strong anti-inflammatory activity. The same results were also obtained when subcutaneously 1% carrageenan was introduced in right hind paw of rats and anti-inflammatory action was compared with the orally administered aspirin at the dose of 300 mg/kg. The findings of carrageenan induced inflammation test conforms the activity detected in Formalin tests. Anti-inflammatory potential was found maximum in the root extract. The leaf and stem extract produced less effect but it was also comparable with standard doses of aspirin.

## CONCLUSION

This detailed study concludes that this plant can safely be utilized as an anti-inflammatory agent alone or in formulation.

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