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In vivo anti-inflammatory activity of rhizome of *Drynaria quercifolia* (L.) J. Sm.

Research Article

Greshma P Raj[1*], Shincymol V V[2], Ansary P Y[3], Sara Moncy Oommen[4]

1. P G Scholar, 2. Associate Professor, 3. Professor & HOD, Department of Dravyaguna Vijnanam, Government Ayurveda College, Tripunithura, Ernakulam, Kerala, India.
4. Professor & HOD, Department of Dravyaguna Vijnanam, Govt. Ayurveda College, Kannur, Kerala, India.

Abstract

Inflammation is found as a common pathogenic process in many of the chronic diseases. To manage them drugs having multiple active principles is needed. Here comes the importance of easily available, potent herbal medicines. *Drynaria quercifolia* (L.) J. Sm., is a medicinal fern and its rhizome is an ingredient of some Ayurvedic formulations with inflammation. Through present study in vivo analysis of powder (*choorna*) of the drug were done. Wistar Albino rats were used for the purpose. One group was control (no treatment) and the other three were treated groups given with the suspension of powdered drug in half the calculated effective dose (0.108 gm/200 gm b. Wt.), calculated effective dose (0.216gm/200 gm b. Wt.) and double the calculated effective dose (0.432 gm/200 gm b. Wt.). Repeated measures ANNOVA with Tukey's post hoc analysis was used as statistical tool to analyse results within the group and one way ANNOVA with Tukey's post hoc analysis was used for between group analysis. In all the three treated groups significant reduction in paw oedema noted at 2nd hour and maximum reduction at 4th hour after drug administration. But a highly significant reduction in paw oedema at shortest time after drug intake was produced by group administered with double the calculated effective dose of the drug. This showed dose dependent anti-inflammatory action of powder of rhizome of *Drynaria quercifolia* (L.) J. Sm. This activity can be due to the presence of anti-inflammatory phytoconstituents in the rhizome such as naringin, quercetin, friedelin, betasitosterol, etc.

Key Words: Inflammation, *Ayurvedic, Fern, Herbal, Choorna, Naringin*.

Introduction

The prevalence of chronic diseases that leads to morbidity and mortality is increasing now a days. Most of such disease are not single entities as they exists with several etiological factors and different pathological process (1). Common pathogenesis common in many chronic diseases is inflammation (2). Drugs having multiple active principles is the best option in order to tackle the multiple target and that therapies should be in a balanced and personalized to avoid adverse reactions (1). Here comes the importance of Ayurveda and its holistic approach in disease management.

In Ayurvedic system of medicine, diseases management is mainly by utilizing the medicinal power of plants and most of them belongs to gymnosperm and angiosperm divisions. But, over exploitation makes their scarcity and reduced land availability makes difficulty in cultivating them. So scientific exploration of therapeutic potential of other divisions like pteridophytes is need of the time. *Drynaria quercifolia* (L.) J. Sm. is such an important pteridophyte having medicinal value described Hortus Malabaricus, a compilation work on medicinal plant wealth of Kerala (3). In northern part of Kerala the plant is known by the name *Thudinthappala* and its rhizome is being used as ingredient of formulations of external application like *Ellumnisadi choorna* (4). Use of its rhizome as food and medicine among tribal in different parts of the country also reported (5).

Antioxidant (6), antiarthritic (7), antipyretic (8), antibacterial (9) and anti-inflammatory (10) activities of various extracts of the rhizome was also evaluated through in vitro and in vivo methods. But the evaluation of the drug in Ayurvedic dosage forms is not available. So the present study aimed on in vivo analysis of anti-inflammatory activity of orally administered rhizome powder (*choorna*) of *Drynaria quercifolia* (L.) J. Sm.

Materials and Methods

Materials

12 female and 12 Male Wistar Albino rats weighing 150 to 200 gm, suspension of the drug, Formalin, Plethysmometer, feeding bottles, beaker, weighing machine, permanent marker, feeding cannula, rat cages, syringes 1 ml.

Procurement of animals

From the proposed source, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala (Reg. No. 328/GO/Re/S//01/CPSEA) 12 female and 12 male Wistar Albino rats were procured. They were acclimatized by keeping in animal house of

*** Corresponding Author:**
Greshma P Raj
PG Scholar,
Department of Dravyaguna vijnanam,
Governemnt Ayurveda College, Tripunithura,
Ernakulam, Kerala, India.
Email Id: greshmapraj@gmail.com

Department of Dravyagunavijnanam, Government Ayurveda College, Tripunithura for 7 days before use.

Dose fixation

There is no classical reference available regarding the dose of *choorna* (powder) of rhizome *Drynaria quercifolia* (L.) J. Sm. As per the references in *Sarangadhara Samhita,* 12 gm is the dose of *choorna* (powder) for adult (11). Using Paget and Barnes table, the effective dose of the powdered drug for rats was calculated (conversion factor 0.018 for 200 gm body weight rats) (12) .0.216 gm was found as the calculated effective dose for rat of 200 gm body weight. Dose of Formalin used was 0.05 ml.

Preparation of *choorna* (powder) for the study

A suspension of the drug was prepared by mixing 12 gm powder in 100 ml of distilled water. So 0.12 gm of the powdered drug was available in 1 ml of suspension. The volume of suspension administered to a rat of 200 gm body weight in half the calculated effective dose, calculated effective dose and double the calculated effective dose was 0.9 ml, 1.8 ml and 3.6 ml respectively.

Mode of administration

Through oral route suspension of the powdered drug was administered to the rats. Formalin was administered in the form of sub cutaneous injection in the left hind limb paw of the rats.

Dosing schedule

Single time administration was done for all the groups through oral route.

Grouping of animals

24 Wistar Albino rats were divided into 4 groups viz Group A (Control- no treatment), Group B (half the calculated effective dose group), C (calculated effective dose group) and D (double the calculated effective dose group). Each group contained 6 rats (3 females, 3 males). (***Table 1***)

Table 1: Grouping of animals for anti-inflammatory activity

Group	Drug Dose
Group A- control (No treatment)	Distilled water (2ml/200g b. Wt)
Group B- half the calculated effective dose	½ X (0.108gm/200gm b. Wt.)
Group C- the calculated effective dose	X (0.216gm/200gm b. Wt.)
Group D- double the calculated effective dose	2 X (0.432gm/200gm b. Wt.)

Methods: Formalin-induced paw oedema model

Food was withdrawn 12 hours before the start of experiment and resumed after completion of experiment. A mark was made on the left hind paw of the weighed rat just beyond tibio-tarsal junction so that every time the paw was dipped in the water column of plethysmometer up to fixed mark to ensure constant paw volume. To ensure uniform hydration, rats were given with 5ml of distilled water by stomach tubes before the start of experiment. Normal paw volume of left hind limb of rats were noted before formalin injection. 0.05 ml of formalin was injected subcutaneously into left hind limb paw of rat 30 min after the oral administration of water to induce acute inflammation. Rats with a minimum of 0.4 ml increase in the normal paw volume were selected for the study. The non-inflamed right paw served as reference for comparison. The respective doses of suspension of powdered drug was administered orally using feeding cannula to animals in each group. Paw volume were recorded at every 1 hour up to 4th hour after drug administration. Changes in paw volume by comparing the values before and after the administration of the powdered drug and the values of the control with the treated groups were used for statistical analysis.

Ethics

Approval from the institutional animal ethics committee was obtained and the number was No B4/2601/2017/AVC.

Results

A comparison was done in paw volume in Group A (Control (no treatment)), Group B (Half the calculated effective dose), Group C (Calculated effective dose) and Group D (Double the calculated effective dose) between before drug administration (BDA) and after drug administration at every 1 hour up to 4th hour. Paw volume was again compared between every 1 hour up to 4th hour separately in all the above groups. The difference in paw volume was also compared between all the groups at before drug administration and after drug administration at every 1 hour up to 4th hour.

In Group A (Control (no treatment)) a highly significant increase in paw volume was noted after the administration of distilled water compared to the paw volume before distilled water administration. (***Table 2***)

Table 2: Comparison of paw volume within Group A (Control (no treatment))

Group A	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st hr	-0.1167	6.931	Yes	***	-0.1879 to -0.04543
BDA Vs 2nd hr	-0.2000	11.88	Yes	***	-0.2712 to -0.1288
BDA Vs 3rd hr	-0.3167	18.81	Yes	***	-0.3879 to -0.2454
BDA Vs 4th hr	-0.4167	24.75	Yes	***	-0.4879 to -0.3454
1st hr Vs 2nd hr	-0.08333	4.951	Yes	*	-0.1546 to -0.01210
1st hr Vs 3rd hr	-0.2000	11.88	Yes	***	-0.2712 to -0.1288
1st hr Vs 4th hr	-0.3000	17.82	Yes	***	-0.3712 to -0.2288
2nd hr Vs 3rd hr	-0.1167	6.931	Yes	***	-0.1879 to -0.04543
2nd hr Vs 4th hr	-0.2167	12.87	Yes	***	-0.2879 to -0.1454
3rd hr Vs 4th hr	-0.1000	5.941	Yes	**	-0.1712 to -0.02876

In Group B (Half the calculated effective dose) and Group C (Calculated effective dose) paw volume reduced significantly after drug administration at 3rd and 4th hour compared to before drug administration. But in Group B at 1st hour a significant increase in paw volume occurred and at 2nd hour an insignificant reduction in the paw volume occurred compared to before drug administration. In Group C at 1st hour after drug administration paw volume remain unchanged and from 2nd hour onwards significant reduction in paw volume noticed when compared to before drug administration. (***Table 3***), (***Table 4***)

Table 3: Comparison of paw volume within Group B (Half the calculated effective dose)

Group B	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st hr	-0.08333	4.385	Yes	*	-0.1638 to -0.002913
BDA Vs 2nd hr	0.0	0.0	No	ns	-0.08042 to 0.08042
BDA Vs 3rd hr	0.1167	6.139	Yes	**	0.03625 to 0.1971
BDA Vs 4th hr	0.1333	7.016	Yes	***	0.05291 to 0.2138
1st hr Vs 2nd hr	0.08333	4.385	Yes	*	0.002913 to 0.1638
1st hr Vs 3rd hr	0.2000	10.52	Yes	***	0.1196 to 0.2804
1st hr Vs 4th hr	0.2167	11.40	Yes	***	0.1362 to 0.2971
2nd hr Vs 3rd hr	0.1167	6.139	Yes	**	0.03625 to 0.1971
2nd hr Vs 4th hr	0.1333	7.016	Yes	***	0.05291 to 0.2138
3rd hr Vs 4th hr	0.01667	0.8771	No	ns	-0.06375 to 0.09709

Table 4: Comparison of paw volume within Group C (Calculated effective dose)

Group C	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st hr	0.0	0.0	No	ns	-0.08696 to 0.08696
BDA Vs 2nd hr	0.1000	4.867	Yes	*	0.01304 to 0.1870
BDA Vs 3rd hr	0.2000	9.733	Yes	***	0.1130 to 0.2870
BDA Vs 4th hr	0.2833	13.79	Yes	***	0.1964 to 0.3703
1st hr Vs 2nd hr	0.1000	4.867	Yes	*	0.01304 to 0.1870
1st hr Vs 3rd hr	0.2000	9.733	Yes	***	0.1130 to 0.2870
1st hr Vs 4th hr	0.2833	13.79	Yes	***	0.1964 to 0.3703
2nd hr Vs 3rd hr	0.1000	4.867	Yes	*	0.01304 to 0.1870
2nd hr Vs 4th hr	0.1833	8.922	Yes	***	0.09637 to 0.2703
3rd hr Vs 4th hr	0.08333	4.056	No	ns	-0.003626 to 0.1703

Table 5: Comparison of paw volume within Group D (Double the calculated effective dose)

Group D	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st hr	0.01667	0.8058	No	ns	-0.07086 to 0.1042
BDA Vs 2nd hr	0.1167	5.641	Yes	**	0.02914 to 0.2042
BDA Vs 3rd hr	0.2333	11.28	Yes	***	0.1458 to 0.3209
BDA Vs 4th hr	0.3000	14.50	Yes	***	0.2125 to 0.3875
1st hr Vs 2nd hr	0.1000	4.835	Yes	*	0.01247 to 0.1875
1st hr Vs 3rd hr	0.2167	10.48	Yes	***	0.1291 to 0.3042
1st hr Vs 4th hr	0.2833	13.70	Yes	***	0.1958 to 0.3709
2nd hr Vs 3rd hr	0.1167	5.641	Yes	**	0.02914 to 0.2042
2nd hr Vs 4th hr	0.1833	8.864	Yes	***	0.09580 to 0.2709
3rd hr Vs 4th hr	0.06667	3.223	No	ns	-0.02086 to 0.1542

Difference in paw volume when compared between various groups at before drug administration and at 1st hour after drug administration were found insignificant (***Table 6***) (***Table 7***).

Table 6: Comparison of paw volume between Groups before drug administration

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.0	0.0	No	ns	-0.1675 to 0.1675
Group A Vs Group C	-0.03333	0.7875	No	ns	-0.2009 to 0.1342
Group A Vs Group D	-0.01667	0.3937	No	ns	-0.1842 to 0.1509
Group B Vs Group C	-0.03333	0.7875	No	ns	-0.2009 to 0.1342
Group B Vs Group D	-0.01667	0.3937	No	ns	-0.1842 to 0.1509
Group C Vs Group D	0.01667	0.3937	No	ns	-0.1509 to 0.1842

Table 7: Comparison of paw volume between Groups at 1st hour

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.03333	0.8687	No	ns	-0.1185 to 0.1852
Group A Vs Group C	0.08333	2.172	No	ns	-0.0685 to 0.2352
Group A Vs Group D	0.1167	3.041	No	ns	-0.0352 to 0.2685
Group B Vs Group C	0.05000	1.303	No	ns	-0.1019 to 0.2019
Group B Vs Group D	0.08333	2.172	No	ns	-0.0685 to 0.2352
Group C Vs Group D	0.03333	0.8687	No	ns	-0.1185 to 0.1852

Difference in paw volume at 2nd hour, 3rd hour and 4th hour after drug administration when compared between various groups found highly significant between Group A (control) with all three treated groups. Comparison between treated groups were insignificant in all these time intervals. (***Table 8***) (***Table 9***) (***Table 10***).

Table 8: Comparison of paw volume between Groups at 2nd hour

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.2000	5.477	Yes	**	0.05547 to 0.3445
Group A Vs Group C	0.2667	7.303	Yes	***	0.1221 to 0.4112
Group A Vs Group D	0.3000	8.216	Yes	***	0.1555 to 0.4445
Group B Vs Group C	0.06667	1.826	No	ns	-0.07786 to 0.2112
Group B Vs Group D	0.1000	2.739	No	ns	-0.04453 to 0.2445
Group C Vs Group D	0.03333	0.9129	No	ns	-0.1112 to 0.1779

At 3rd hour and 4th hour after drug administration, the paw volume when compared between the various groups shown high significance between Group A and Group B, Group A and Group C, between Group A and Group D ($p<0.001$) (***Table 9***) (***Table 10***).

Table 9: Comparison of paw volume between Groups at 3rd hour

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.4333	13.80	Yes	***	0.3090 to 0.5576
Group A Vs Group C	0.4833	15.39	Yes	***	0.3590 to 0.6076
Group A Vs Group D	0.5333	16.98	Yes	***	0.4090 to 0.6576
Group B Vs Group C	0.05000	1.592	No	ns	-0.07429 to 0.1743
Group B Vs Group D	0.1000	3.184	No	ns	-0.02429 to 0.2243
Group C Vs Group D	0.05000	1.592	No	ns	-0.07429 to 0.1743

Table 10: Comparison of paw volume between Groups at 4th hour

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.5500	13.53	Yes	***	0.3891 to 0.7109
Group A Vs Group C	0.6667	16.40	Yes	***	0.5058 to 0.8276
Group A Vs Group D	0.7000	17.22	Yes	***	0.5391 to 0.8609
Group B Vs Group C	0.1167	2.870	No	ns	-0.04424 to 0.2776
Group B Vs Group D	0.1500	3.690	No	ns	-0.01091 to 0.3109
Group C Vs Group D	0.03333	0.8199	No	ns	-0.1276 to 0.1942

Discussion

In control group of anti-inflammatory study a highly significant increase in paw volume was observed at 1st hour after the administration of distilled water compared to the paw volume before administration of distilled water ($p<0.001$). Similar observations were also noted at 2nd, 3rd and 4th hour after distilled water administration ($p<0.001$). So in control group a marked increase in paw volume was observed at every hour in the study. Within half the calculated effective dose group, after the administration of drug (0.108 gm/ 200 gm body weight) paw volume increased significantly ($p<0.05$) at the 1st hour compared to the paw volume before drug administration. In 2nd hour paw volume reduced insignificantly ($p>0.05$) and became same as the value of before drug administration. There after the paw volume reduced gradually with high significance at 3rd hour ($p<0.01$) and at 4th hour ($p<0.001$). This showed that the drug administered in half the calculated effective dose produced a favourable anti-inflammatory action at 3rd hour after administration and its maximum anti-inflammatory action was at 4th hour after administration compared to before drug administration. After the administration of calculated effective dose (0.216 gm/200 gm body weight) paw volume remained unchanged at the first hour ($p>0.05$). At 2nd hour after drug administration the reduction in paw volume was significant ($p<0.05$) compared to before drug administration. There after the reduction in paw volume was gradual with high significance at 3rd and at 4th hour after drug administration compared to the paw volume before drug administration ($p<0.001$). Comparison between the reduced paw volume within the group at 3rd and 4th hour after drug administration was insignificant ($p>0.05$). This showed that the drug administered in calculated effective dose produced its anti-inflammatory effect at 2nd hour after administration and maximum effect was at 3rd and at 4th hour after administration compared to before drug administration. Within double the calculated effective dose group, after administration of drug (0.432 gm/200 gm body weight) paw volume reduced gradually in every 1 hour up to 4th hour compared to the paw volume before drug administration. The reduction in paw volume was insignificant ($p>0.05$) at 1st hour and then it became highly significant at 2nd hour ($p<0.01$), at 3rd hour ($p<0.001$) and at 4th hour ($p<0.001$) when compared with paw volume before drug administration. Comparison between the reduced paw volume within the group at 3rd and 4th hour after drug administration was insignificant ($p>0.05$). So that the drug in double the calculated effective dose produced a favourable action at 2nd hour after administration and maximum anti-inflammatory action at 3rd and at 4th hour after drug administration compared to before drug administration. In experimental animals a highly significant ($p<0.01$) reduction in paw volume at the shortest time period (2nd hour) occurred after

administration of the double the calculated effective dose. In all these treated groups a maximum reduction in paw volume with high significance ($p<0.001$) noted at 4th hour after drug administration compared to the paw volume before drug administration. When the difference in paw volume compared between various doses at 1st, 2nd, 3rd and 4th hour after drug administration, insignificant results obtained at 1st hour after drug administration. When the difference in paw volume of half the calculated effective dose group compared with the control group highly significant result seen at 2nd hour ($p<0.01$), at 3rd hour ($p<0.001$) and at 4th hour ($p<0.001$) after drug administration. When the difference in paw volume in control group compared with calculated effective dose and double the calculated effective dose a highly significant reduction in inflammation was obtained at 2nd, at 3rd and at 4th hour after drug administration in ($p<0.001$). When the difference in paw volume in calculated effective dose group compared with double the calculated effective dose at 3rd hour after drug administration the significance level was found high ($p<0.001$). These comparisons showed that the double the calculated effective dose is the most effective dose of *choorna* (powder) of rhizome of *Drynaria quercifolia* (L.) J. Sm. to produce an anti-inflammatory action. An immediate and sustained reduction in inflammation with high significance was shown by the groups treated with double the calculated effective dose.

Rhizome of the plant contains flavonoids like naringin and quercetin, triterpenoids like friedelin and β- amyrin, alkaloids, proteins, saponins, etc (5). The process of inflammation will be reduced by naringin by reducing the expression of signalling factors like interleukin-6 (IL-6), interleukin-8 (IL-8), nuclear factor erythroid 2-related factor 2 (Nrf2), inducible nitric oxide synthase (iNOS), and TNF-α (13).Quercetin inhibits lipopolysaccharide (LPS)-induced TNF-α production in macrophages and inhibits production of cyclooxygenase (COX) and lipoxygenase (LOX) (14). Friedelin can inhibition the synthesis of prostaglandin (15).By suppressing the release of TNF-α or its pro-inflammatory action β-amyrin acts as an anti-inflammatory agent (16).Beta sitosterol can inhibits the infiltration of inflammatory cells, and the levels of interleukin-4, IgE and histamine in serum and thus act as an anti-inflammatory agent (17).

Conclusion

The present study point toward the dose dependent anti-inflammatory activity of internally administered choorna (powder) of rhizome of Drynaria quercifolia (L.) J. Sm. This activity can be due to the presence of anti-inflammatory phytoconstituents like naringin, quercetin, friedelin, betasitosterol, etc. in the rhizome of the plant.

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