IJAYUSH
International Journal of AYUSH
AYURVEDA, YOGA, UNANI, SIDDHA AND HOMEOPATHY
http://internationaljournal.org.in/journal/index.php/ijayush/

International Journal
Panacea
Research library
ISSN: 2349 7025

Original Research Article | Volume 10 Issue 5 | Sept-Oct 2021

EXPERIMENTAL EVALUATION OF ANTI-PYRETIC ACTIVITY OF RHIZOME OF *DRYNARIA QUERCIFOLIA* (L.) J. SM.

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ABSTRACT

Plants are being used as a source of medicine since ancient times. But only a limited number of studies were done to explore the medicinal power of pteridophytes like *Drynaria quercifolia* (L.) J. Sm. *Ayurvedic* physicians in Kerala using its rhizome externally to cure rheumatic complaints, fissure in ano etc. Reports shows that the tribal peoples also uses its rhizome as food and medicine. Present study aimed on revalidating the medicinal property of internally administered rhizome for its antipyretic action thorough in-vivo method. Suspension of the rhizome powder was orally given to Wistar Albino rats in half the calculated effective dose (0.108gm/200gm b. Wt.), calculated effective dose (0.216gm/200gm b. Wt.) and double the calculated effective dose (0.432gm/200gm b. Wt.) and the results were analyzed using repeated measures ANNOVA with Tukey's post hoc analysis within the groups and one way ANNOVA with Tukey's post hoc analysis for between group. All the three treated groups shown reduction in rectal temperature but the drug administered in half the calculated effective dose shown maximum antipyretic effect at shortest time after administration that was sustainable. Study revealed dose dependent anti-pyretic activity of *choorna* (powder) of rhizome of *Drynaria quercifolia* (L.) J. Sm.

Key words: *Drynaria quercifolia* (L.) J. Sm., pteridophytes, rhizome, *Ayurvedic,* anti-pyretic

INTRODUCTION

Plants are being used as a source of medicine since ancient times. Various researches are being carried out in medicinal plants to explore their therapeutic potential. But most of such researches are confined in angiosperm or gymnosperm plants. And only limited number of studies were done to explore the medicinal power of pteridophytes. [1]*Drynaria quercifolia* (L.) J. Sm. is such a medicinal pteridophyte from Polypodiaceae family known as *Pannalkizhangu* or *Thudinthappala* in Malayalam.

Ayurvedic physicians in Kerala is using its rhizome as external application in the management of rheumatic complaints (*vataraktam*), [2] fissure in ano (*bhagandaram*), [3] diabetic carbuncle (*prameha pidaka*), [4] etc. *Hortus malabaricus* of 17th century, a compilation work on plant wealth of *Malabar* region of Kerala give description on identification features and medicinal uses of the plant. [5] *Attukal* soup is preparation popular among the Tribals of Kalakad-Mundanthurai Tiger Reserve, Tamilnadu for getting relief from rheumatic complaints is prepared out of the rhizome of this plant.[6]

Previous researches on extracts of this drug showed its potential as an analgesic, anti-inflammatory, antipyretic, anti-microbial agent. [6]There is no researches on any of the dosage forms mentioned in *Ayurvedic* classics for this plant and its efficacy while taking internally. So the present study aimed on evaluating the antipyretic activity of orally administered powder (*choorna*) of rhizome of *Drynaria quercifolia* (L.) J. Sm.

EXPERIMENTAL

A. Material

1. Materials used

12 Male and12 female Wistar Albino rats weighing 150 to 200 gm, suspension of the powdered drug, 5% Dextrose, Brewer's yeast, weighing machine, Digital thermometer, feeding bottles, beaker , permanent marker, feeding cannula, syringes 1 ml, 2 ml and 5 ml, gloves, needles (22 and 24 gauge), rat cages.

2. Procurement of animals

A total of 24 Wistar Albino rats 12 female and 12 male were procured from the proposed source, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. (Reg. No. 328/GO/Re/S//01/CPSEA). The animals were kept in animal house of Department of Dravyaguna vijnanam, Government Ayurveda College, Tripunithura and acclimatized for standard laboratory conditions for 7 days before use.

3. Dose fixation of *choorna* (powder) of the drug

Drynaria quercifolia (L.) J. Sm. is an extra-pharmacopoeial drug. So classical reference regarding the dose of *choorna* (powder) of its rhizome is not available. 12 gm is the human adult dose of *choorna* (powder) as per references available in *Sarangadhara Samhita*. [7] Therapeutic dose to rat was calculated on the basis of body surface area ratio given in Paget and Barnes table (conversion factor 0.018 for 200 gm body weight rats). So the calculated effective dose of this drug for 200 gm weight rat was found as 0.216 gm.

4. Preparation of *choorna* (powder) for the study

By considering 12 gm as the human dose, a suspension is prepared by mixing the same amount in 100 ml of distilled water. So 1ml of the suspension contains 0.12 gm of the powdered drug and the volume to be administered to each animal were calculated according to weight of the animal.

5. Preparation of yeast solution

By adding 5 gm of Dextrose in 100 ml distilled water5% Dextrose was prepared.1.5 gm of Brewer's yeast was added to the 10 ml of 5% Dextrose to prepare 15 % of yeast solution. The solution get activated by keeping closed for about 15 minutes.

6. Mode of administration

Suspension of the powdered drug administered thorough oral route and the Brewer's yeast was administered as sub cutaneous injection.

7. Dosing schedule

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

8. Grouping of animals

The animals were divided into 4 groups of 6 rats (3 males, 3 females) each. Group A (Control) received distilled water. Group B, C and D were the treated groups received half the calculated effective dose, calculated effective dose and double the calculated effective dose of the *choorna* (powder) of the drug respectively. [Table 1]

Table 1: Grouping of animals for anti-pyretic activity

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

B. Methods: Brewer's yeast induced pyrexia model

Rectal temperature of rats were noted by inserting the thermo- sensitive end of digital thermometer into the rectum and keeping it there for 1 min. Then 15% of Brewer's yeast solution in doses of 10 ml/kg body weight were injected subcutaneously in the back below the nape of neck of the rat and in order to spread the suspension beneath the skin the site of injection was massaged thereafter. Food was withdrawn immediately after yeast administration and the room temperature was maintained between 22°C and 26° C. After 18 hours, animals with 1°F rise in the basal temperature were selected for the study and

were given with the respective doses in all the groups. After drug administration, rectal temperature was noted hourly for 3 hours.

Figure 1: Subcutaneous injection of Brewer's yeast

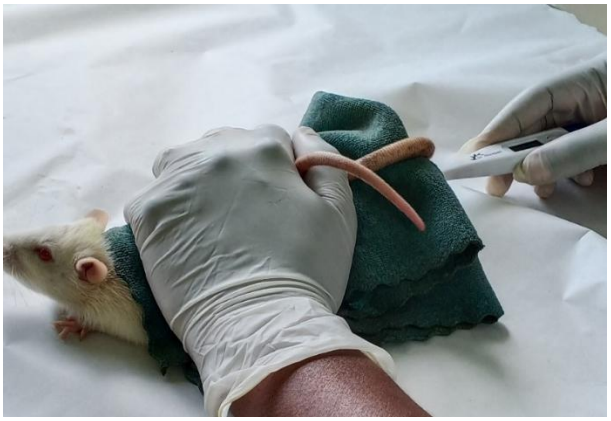

Figure 2: Measuring rectal temperature of Wistar Albino rat

Ethics

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

RESULTS

Statistical analysis within the group was done using repeated measures ANOVA with Tukey's post hoc analysis and between groups using one way ANNOVA with Tukey's post hoc analysis. A comparison was done in rectal temperature in Group A (Control), 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 3rd hour. Rectal temperature was again compared between after drug administration at every 1 hour up to 3rdhour separately in all the above groups. The difference in rectal temperature was also compared between all the groups at before drug administration and after drug administration at every 1 hour up to 3rd hour.

After the administration of distilled water in Group A (Control - no treatment), the rectal temperature raised gradually with significance at every one hour up to 3rd hour of assessment. [Table 2]

Table 2: Comparison of rectal temperature within Group A

Group A	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1sthour	-0.2667	4.229	Yes	*	-0.5237 to -0.009629
BDA Vs 2ndhour	-0.4000	6.343	Yes	**	-0.6570 to -0.1430
BDA Vs 3rd hour	-0.5833	9.250	Yes	***	-0.8404 to -0.3263
1st hour Vs 2nd hour	-0.1333	2.114	No	ns	-0.3904 to 0.1237
1st hour Vs 3rd hour	-0.3167	5.021	Yes	*	-0.5737 to -0.05962
2nd hour Vs 3rd hour	-0.1833	2.907	No	ns	-0.4404 to 0.07371

In Group B (Half the calculated effective dose) the reduction in rectal temperature before drug administration and after drug administration was found highly significant at all the time intervals. [Table 3]

Table 3: Comparison of rectal temperature within Group B

Group B	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st hour	0.4667	5.262	Yes	**	0.1052 to 0.8282
BDA Vs 2nd hour	0.6833	7.705	Yes	***	0.3218 to 1.045
BDA Vs 3rd hour	1.000	11.28	Yes	***	0.6385 to 1.361

1st hour Vs 2nd hour	0.2167	2.443	No	ns	-0.1448 to 0.5782
1st hour Vs 3rd hour	0.5333	6.014	Yes	**	0.1718 to 0.8948
2nd hour Vs 3rd hour	0.3167	3.571	No	ns	-0.04483 to 0.6782

The comparison on reduction in rectal temperature in Group C (Calculated effective dose) and Group D (Double the calculated effective dose) between before drug administration and after drug administration at every 1 hour up to 3rd hour was highly significant at 2nd and 3rd hour and significant at 1st hour. [Table 4], [Table 5]

Table 4: Comparison of rectal temperature within Group C

Group C	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st hour	1.017	4.555	Yes	*	0.1070 to 1.926
BDA Vs 2nd hour	1.333	5.974	Yes	**	0.4236 to 2.243
BDA Vs 3rd hour	1.567	7.020	Yes	***	0.6570 to 2.476
1st hour Vs 2nd hour	0.3167	1.419	No	ns	-0.5930 to 1.226
1st hour Vs 3rd hour	0.5500	2.464	No	ns	-0.3597 to 1.460
2nd hour Vs 3rd hour	0.2333	1.045	No	ns	-0.6764 to 1.143

Table 5: Comparison of rectal temperature within Group D

Group D	Mean Diff.	q	Significance	Summary	95% CI of diff
BDA Vs 1st	1.317	4.352	Yes	*	0.08347 to 2.550

hour					
BDA Vs 2nd hour	1.950	6.445	Yes	**	0.7168 to 3.183
BDA Vs 3rd hour	2.067	6.831	Yes	**	0.8335 to 3.300
1st hour Vs 2nd hour	0.6333	2.093	No	ns	-0.5999 to 1.867
1st hour Vs 3rd hour	0.7500	2.479	No	ns	-0.4832 to 1.983
2nd hour Vs 3rd hour	0.1167	0.3856	No	ns	-1.117 to 1.350

The difference in rectal temperature before drug administration when compared between the four groups (control and the three treated groups) were found insignificant. [Table 6]

Table 6: Comparison of rectal temperature between Groups before drug administration

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	-0.1167	0.2990	No	ns	-1.661 to 1.428
Group A Vs Group C	0.5167	1.324	No	ns	-1.028 to 2.061
Group A Vs Group D	0.3000	0.7688	No	ns	-1.244 to 1.844
Group B Vs Group C	0.6333	1.623	No	ns	-0.9111 to 2.178
Group B Vs Group D	0.4167	1.068	No	ns	-1.128 to 1.961

Group C Vs Group D	-0.2167	0.5553	No	ns	-1.761 to 1.328

At 1st hour, 2nd hour and 3rd hour after drug administration, a highly significant difference in rectal temperature were noted between Group A (control) and Group C (calculated effective dose) and betweenGroup A (control) and Group D (double the calculated effective dose) .[Table 7], [Table 8], [Table 9]

Table 7: Comparison of rectal temperature between Groups at 1st hour after drug administration

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.6167	1.828	No	ns	-0.7182 to 1.952
Group A Vs Group C	1.800	5.337	Yes	**	0.4651 to 3.135
Group A Vs Group D	1.883	5.584	Yes	**	0.5484 to 3.218
Group B Vs Group C	1.183	3.509	No	ns	-0.1516 to 2.518
Group B Vs Group D	1.267	3.756	No	ns	-0.06825 to 2.602
Group C Vs Group D	0.08334	0.2471	No	ns	-1.252 to 1.418

Table 8: Comparison of rectal temperature between Groups at 2nd hour after drug administration

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	0.9667	2.578	No	ns	-0.5174 to 2.451

Group A Vs Group C	2.250	6.001	Yes	**	0.7660 to 3.734
Group A Vs Group D	2.650	7.068	Yes	***	1.166 to 4.134
Group B Vs Group C	1.283	3.423	No	ns	-0.2007 to 2.767
Group B Vs Group D	1.683	4.490	Yes	*	0.1993 to 3.167
Group C Vs Group D	0.4000	1.067	No	ns	-1.084 to 1.884

Table 9: Comparison of rectal temperature between Groups at 3rd hour after drug administration

Groups	Mean Diff.	q	Significance	Summary	95% CI of diff
Group A Vs Group B	1.467	3.950	No	ns	-0.002850 to 2.936
Group A Vs Group C	2.667	7.182	Yes	***	1.197 to 4.136
Group A Vs Group D	2.950	7.946	Yes	***	1.480 to 4.420
Group B Vs Group C	1.200	3.232	No	ns	-0.2695 to 2.670
Group B Vs Group D	1.483	3.995	Yes	*	0.01381 to 2.953
Group C Vs Group D	0.2833	0.7631	No	ns	-1.186 to 1.753

DISCUSSION

Within the control group of anti-pyretic activity, after the administration of distilled water (2ml/ 200 gm body weight) rectal temperature was found increased with significance at every one up to 3rd when compared with rectal temperature before distilled

water administration. Rectal temperature reduced with high significance after the drug administration in half the calculated effective dose (0.108 gm/ 200 gm body weight) at 1st hour ($p<0.01$), at 2nd and 3rd hour ($p<0.001$) compared to rectal temperature before drug administration. The reduction in rectal temperature after drug administration at 2nd and 3rd hour within the group was insignificant ($p>0.05$). So drug in half the calculated effective dose had antipyretic action from 1st hour after drug administration and maximum effect at 2nd and at 3rd hour after drug administration. Within the calculated effective dose group, after administration of drug (0.216 gm/200 gm body weight) rectal temperature reduced gradually with significance at 1st hour ($p<0.05$) and with high significance at 2nd hour ($p<0.01$) and at 3rd hour ($p<0.001$) compared to rectal temperature before drug administration. So antipyretic effect of calculated effective dose group starts at 1st hour and maximum at 3rd hour. Rectal temperature reduced with significance after the administration of double the calculated effective dose (0.432 gm/200gm body weight) at 1st hour ($p<0.05$) and with high significance at 2nd and 3rd hour ($p<0.01$). The reduction in rectal temperature after drug administration at 2nd and 3rd hour within the group was insignificant ($p>0.05$). So drug in this dose had antipyretic activity at 1st hour and maximum at 2nd and at 3rd hour after drug administration. Even though all the three treated groups started showing its antipyretic effect after drug administration a highly significant result ($p<0.01$) within short time (1st hour) that sustained at 2nd and at 3rd hour was seen in half the calculated effective dose group. At 3rd hour, all the three treated groups showed maximum antipyretic effect. When difference in rectal temperature compared between control group and half the calculated effective dose group insignificant ($p<0.05$) results were obtained at 1st, 2nd and 3rd hour after drug administration. The difference in rectal temperature at 1st hour after drug administration when compared between control group and calculated effective dose group, a highly significant ($p<0.01$) result was found. Similar results were seen on comparison between control group and double the calculated effective dose group at 1st hour after drug administration and the comparison between control and calculated effective dose group at 2nd hour after drug administration. A highly significant result with $p<0.001$ was obtained in comparison between control group and calculated

effective dose group at 3rd hour after drug administration and control and double the calculated effective dose group at 2nd and 3rd hour. When reduced rectal temperature of half the calculated effective dose compared with double the calculated effective dose significance was found at 2nd and 3rd hour ($p<0.05$). These comparisons shows that after the administration of *choorna* (powder) of rhizome of *Drynaria quercifolia* (L.) J. Sm. an immediate and sustainable reduction in rectal temperature with high significance was produced by the drug at its half the calculated effective dose. The drug starts acting as an anti-pyretic drug at 1st hour after administration and the maximum antipyretic effect was at 3rd hour after administration.

Rhizome of this plant contain phyoconstituents such as flavonoids, saponins, alkaloids, betasitosterol, triterpenoids, etc. Flavonoids decrease the release of arachidonic acid, that leads to suppression of the inflammatory mediators such as prostaglandins and lipoxygenase, the end products responsible for fever. [8] Naringin, naringenin and quercetin are the main flavonoids in this rhizome. By reducing the production of prostaglandin (PGE_2), IL-6 (Interleukin- 6), NO (Nitric Oxide) and TNF-α (Tumor Necrosis Factor- α), naringin helps in fever reduction. [9] Beta-sitosterol reduces the synthesis of prostaglandin and leukotriene, by forming essential polyunsaturated fatty acids from linoleic acid (linoleic acid is required for prostaglandin and leukotriene synthesis). Secretion of TNF-αand pro-inflammatory cytokines were also reduced by it. By stimulating the innate immune system improvement in overall health was obtained. [10] Friedelin can influence the biosynthesis of prostaglandin and thus helps in reducing the elevated temperature. [11]

CONCLUSION

In anti-pyretic activity an immediate and sustainable reduction in rectal temperature with high significance was demonstrated by the drug administered in half the calculated effective dose (0.108gm/200gm body weight). The drug started acting as an anti-pyretic agent at 1st hour with high significance ($p<0.01$) and showed a maximum antipyretic effect at 2nd hour ($p<0.001$) and effect sustained thereafter at 3rd hour with half the calculated

effective dose. This point towards the dose dependent anti-pyretic activity of *choorna* (powder) of rhizome of *Drynariaquercifolia* (L.) J. Sm.

ACKNOWLEDGMENT

I am immensely grateful to my guide, Dr. Shincymol V. V. MD (Ay), Associate Professor, Department of Dravyaguna vijnanam, Govt. Ayurveda College, Tripunithura, for her timely and careful guidance and suggestions to bring in excellence in this work. I am thankful to Dr. P Y Ansary MD, PhD (Ay), Professor & HOD, Department of Dravyaguna vijnanam, Govt. Ayurveda College, Tripunithura for his expert guidance, careful suggestions advices and encouragement throughout this work. I am grateful to Dr. Sara Moncy Oommen MD (Ay), Professor& HOD, Department of Dravyaguna vijnanam, Govt. Ayurveda College, Kannur for her meticulous guidance, advices and suggestions while doing the work.

CONFLICT OF INTEREST

Nil

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