EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 8
433-436
Year: 2022

EXPERIMENTAL EVALUATION OF HUTABHUGADI CHOORNAM WITH SPECIAL REFERENCE TO ITS ANTI-INFLAMMATORY PROPERTIES

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Article Received on 14/06/2022 **Article Revised on 04/07/2022** **Article Accepted on 24/07/2022**

ABSTRACT
Choorna kalpana is an Upakalpana of Kalka kalpana in which the drugs are made into a fine powder. Hutabhugadi choornam is one such Poly-herbal preparation having ingredients that are readily available at low cost and easy to prepare. This formulation is mentioned in Sahasrayogam, choorna prakarana. It is mainly indicated in the treatment of Agnimandya, Pandu, Shopha and Arsha. Shopha can be correlated to Inflammation. Inflammation is defined as the local response of living mammalian tissue due to any agent. The present study is to evaluate the anti-inflammatory properties of Hutabhugadi Choornam on Wister Albino Rats.

KEYWORDS: Hutabhugadi choornam, Anti-Inflammatory, Wister albino rats.

INTRODUCTION
The Indian Pharmaceutical history is as primitive as the history of Ayurveda. It has flourished and elegantly designed Panchavidha Kashaya kalpanas and Upakalpanas that led to the foundation of hundreds of effective formulations.[1] Upakalpanas came into existence to address the various problems associated with dosage, route of administration etc. Choorna kalpana is an Upakalpana of kalka kalpana[2] in which dried raw drugs are made into fine powder and filtered through a clean cloth. Hutabhugadi choornam is a preparation mentioned in Sahasrayogam and is indicated in the management of Agnimandya, Pandu, Arsha and Shopha.[3] it is given along with amla udishvita. Shopha can be correlated with Inflammation. Inflammation is defined as the local response of living mammalian tissue injury due to any agent. It is the body defence reaction in order to eliminate or limit the spread of injurious agent as well as to improve the consequent necrosed cells and tissues.[4] An experiment is a study that is designed to compare the benefits of an intervention with that of a standard treatment or without treatment. The present study is to evaluate the Anti-Inflammatory property of hutabhugadi choornam on Carrageenan induced inflammation in Wister Albino rats.

MATERIALS AND METHODS
An experiment is a study done to compare the benefits of any intervention with that of standard treatments or no treatment at all, like a new drug therapy or a prevention program, or to elicit the cause and the effect. Such a study is performed prospectively.[5] Laboratory animals are used in experimental studies with animal models as models of humans or other species that are targets.

In the present study, anti-inflammatory effect of Hutabhugadi choornam was evaluated on Albino rats.

MATERIALS
Test drug- Choorna- Hutabhugadi choornam. The choorna for anti-inflammatory study was prepared in accordance to the standard operating procedure. Wister albino rats of either sex were used for the anti-inflammatory studies. The animals were in a group of six animals per cage under standard environmental conditions. They were given rat and mice feed and water. Anti-inflammatory study was approved by CPCSEA approved local ethical committee.

Digital plethysmometer
The digital water Plethysmometer is designed to provide a highly useful tool in the measurement of small paw changes. This test is typically used to follow the evolution of the inflammatory response experimentally

or anti- oedema properties of pharmacological substances.

METHODS

Carrageenan induced paw oedema
Anti- inflammatory effect
There are two methods- In-vitro and In-vivo method.
The prominent In-vitro models of inflammation are Carrageenan induced oedema, Pleural exudation and Cotton pellet implantation. The methods of early pleural exudation and Carrageenan induced oedema assess the efficacy of compounds against transudative and exudative phases of inflammatory reaction respectively.[6] Chemically Carrageenan is a sulfated polysaccharide from seaweed.[7] The well recognized method of Winter et al, 1962 is followed.[8]

Criteria

Table no. 1: Inclusion and Exclusion criteria on selection of rat.

Inclusion criteria	Healthy active rats of both sexes are taken for the study. Wistar albino rats weighing 150- 200gms
Exclusion criteria	Unhealthy Wistar albino rats Weight range below 150g to 200g

Grouping

Sample- 18 Albino rats of both sexes were selected and grouped into 3

Table no. 2: Grouping of rats for carrageenan induced paw oedema.

Group	No of rats	Group name	Medicine
1	6	Control group	Distilled water
2	6	Standard group	Diclofenac
3	6	Test group	Hutabhugadi choornam

Mode of administration of drugs- Internal

Dose selection

Dose selection was done on the basis of body surface area ratio using the table of Paget and Barnes (1964 cited by Ghosh; 1984). It was done as follows.
Human dose X surface area ratio convertibility factor for rat or mouse as required. Conversion of the dose obtained above to dose in mg or g/kg by multiplying with suitable factor based on the average weight of the animal.

Dose for rats

Dose for hutabhugadi choornam

Human dose X 0.018 for rat weighing 200g
i.e 12gm x 0.018 x 5= 1.08g/kg= 1.08mg/g along with takra

Dose of standard drug: Diclofenac

10mg/kg body wt-3 days dosing

Route of drug administration

The test drug was administered according to the body weight of the animals by oral route with the help of gastric cathether. The drug was administered along with Udishvita.

Procedure

The test drug was administered once daily for seven consecutive days. The standard group was administered with drug Diclofenac (10mg/kg). On the 7th day prior to carrageenan injection the initial paw volume of the left hind paw would be measured using a Plethysmometer as follows:One hour after drug administration paw oedema was produced by injecting 0.1 ml freshly prepared 1% carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administered with tap water in the dose of 2ml/100g body weight to ensure uniform hydration. This is supposed to minimize the variation in oedema formation.

The intensity of oedema formation was recorded after 1hr, 3hr, 6hr and 24hr carrageenan injection. Results were expressed as percentage increase in paw volume in comparison to the initial values. Percentage increase in paw volume was calculated by subtracting the initial paw volumes from the paw volumes obtained after the injection of the phlogistic agent. The figure is divided by the initial paw volume and multiplied by hundred.

Statistical analysis

The results of the present study will be analyzed statistically by ANOVA test.

OBSERVATIONS AND RESULTS

The experimental data were expressed as Mean ±SEM. The data was analysed by one way Anova followed by Dunnet multiple t as post Hoc test for determining the level of significance of the observed. A ‘p’ value of less than 0.05 was considered statistically significant. Graph pad in Stat – 3 software was used for statistical analysis of the generated data.

The effect of test drug on paw oedema 1, 3, 6, 24 hours

Table 3: Effect of hutabhugadi choornam on carrageenan induced paw volume in the time interval.

Group	Basal	1st hour	3rd hour	6th hour	24th hour
Control	0.78 ±0.06	1.19 ± 0.10	1.28 ± 0.07	1.46 ± 0.12	1.19 ± 0.10
Standard	0.74 ± 0.03	1.00 ± 0.02	1.31 ± 0.08	0.93 ± 0.01	0.87 ± 0.01
Test	0.72 ± 0.02	1.27 ± 0.03	1.55 ± 0.04	1.03 ± 0.04	0.84 ± 0.01

Data: MEAN ± SEM, *P<0.05, **P<0.01

The effect of Hutabhugadi choornam are depicted in the table. The data shows there was increase in paw volume in the control group of 1st,3rd, 6th hour when compared to the basal volume of control group, the observed increase was found to be statistically significant. The increase in 24th hour was found to be non-significant.

In reference standard group, increase in paw volume was found in 3rd and 6th hour when compared to basal volume of control group, increase is considered to be statistically significant. The increase in 24th hour was found to be non significant.

In test drug group (hutabhugadi), increase in paw volume was seen in 1st, 3rd, 6th hour. The observed increase was found to be statistically very significant when compared to the basal paw volume. The increase in the paw volume in 24th hour was found to be non- significant.

Table 4: Effect of Hutabhugadi choornam on percentage change in paw volume in 1hour after carrageenan injection.

Group	1st hour	% change
Control	54.67 ± 10.56	-
Standard	37.41± 10.05	31.57↓
Test (hutabhugadi)	72.27 ± 9.05	32.18↑

The data shows that there was decrease in the increased percentage of paw volume of standard group in the 1st hour when compared to control group. There was increase in paw volume of test group as compared to control group in 1st hour. The increase was found to be non significant.

Table 5: Effect of hutabhugadi choornam on percentage change in paw volume in 3 hours after carrageenan injection.

Group	3rd hour	% change
Control	66.76 ± 6.93	-
Standard	80.18 ± 16.60	20.10↑
Test(hutabhugadi)	60.46 ± 6.69	9.43↓

Data: MEAN±SEM

The data shows there was increase in paw volume of standard group as compared to control group in the 3rd hour but the increase was found to be statistically non-significant.

Table 6: Effect of Hutabhugadi choornam on percentage change in paw volume in 6 hours after carrageenan injection.

Group	6th hour	% change
Control	90.26 ± 12.60	-
Standard	27.35 ± 7.20	69.69↓
Test(hutabhugadi)	36.68 ± 6.22	59.35↓

Data: MEAN±SEM

The data shows there was decrease in increased paw volume of standard group and test group as compared to control group in 6th hour. The decrease is found to be statistically very significant.

Table 7: Effect of Hutabhugadi choornam on percentage change in paw volume in 24 hours after carrageenan injection.

Group	24th hour	% change
Control	53.93 ± 6.83	-
Standard	20.44 ± 7.41	62.09↓
Test(hutabhugadi)	15.45 ± 3.52	71.35↓

Data: MEAN±SEM

The data shows decrease in increased paw volume of standard group as well as test group as compared to control group in 24th hour. The decrease was found to be statistically very significant.

Both standard and test group showed decrease in percentage change in paw volume when compared to control group in 24 hours

Hutabhugadi choornam shows much decrease in the paw volume when compared to the control group and the decrease is found to be statistically very significant.

DISCUSSION

The study was conducted to evaluate the anti-inflammatory property of Hutabhugadi choornam. For this, the study model was Carrageenan induced paw oedema. The paw oedema was measured based on the Archimedes principle. The choornam was subjected to invivo animal (per se) for an experimental module of anti-inflammatory effect of carrageenan induced inflammation in Wistar Albino rat paw. Experimental data was analyzed using ANOVA followed by Dunnet multiple t-test as POC hoc test if P-value is less than 0.05 with graph pad in Stat-3 software.

From the observation and statistical report of the control group, standard group, and test group (Hutabhugadi choornam), it was found that there is a decrease in the increased paw volume standard group by 31.57% and an increase in paw volume of the test group by 32.18% when compared to control group in the 1st hour. In the 3rd hour, it shows the standard group has increased paw volume to 20.10% when compared to the control group. The test group showed a decrease in 9.43% in the increased paw volume when compared to control group.

In the 6th hour, the standard group showed a 69.69% decrease in the paw volume and the test group also showed a 59.38% decrease in the paw volume. The decrease is found to be statistically very significant.

In the 24th hour, both the standard and the test groups showed a decrease of 68.09% and 71.35% respectively in the paw volume.

The decrease was found to be statistically significant.

Thus it can be observed that Hutabhugadi choornam exhibits good Anti-inflammatory properties.

CONCLUSION

This experimental study includes protocol of the study, dose fixation for Albino rats and observations by statistical analysis on Anti-Inflammatory effect of Hutabhugadi choornam on carrageenan induced paw oedema. Hutabhugadi choornam has shown good Anti-Inflammatory properties.

Limitations of the study

Shopha can either present as a disease by itself or as a symptom of some other ailment. One has to rule out whether this yoga is effective in which condition i.e. shopha as a disease or a symptom like that seen in Pandu roga where ‘akshi kuta shotha’ is a presenting feature.

Inflammation may be acute or chronic. The experimentation on rats was carried out only in acute inflammatory models. Experimentation can only pave a way to shophahara property and this has to be further substantiated with further clinical study.

Scope of the study

A detailed clinical study of Hutabhugadi choornam on different kinds of shopha based on different disease conditions has to be done.

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