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Anti-inflammatory activity of *Sebastiania chamaelea* (L.) Mull.Arg – An experimental study

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Abstract

Inflammation is the immune response raised by the body against an irritant. Severity of the inflammation decides the reaction pronounced by the body. Even the impaired diet can cause inflammatory changes in a human body. Anti-inflammatory drugs are capable of reversing the situation. In the current scenario herb based medicinal preparations are really important for meeting the health care needs. Ayurveda have an inevitable role to play in this scenario And presently there is a need to validate the local flora to meet the rising need for herb based medicinal preparations. As an initial step for the purpose the drug *Sebastiania chamaelea* (L.) Mull.Arg was subjected experimental evaluation of anti-inflammatory activity. The activity using Formalin- induced paw oedema method were done in Wistar albino rats at three different doses. The data were subjected to statistical analysis using one way ANOVA with Tukey's post hoc test. Statistically significant results were obtained for all test groups when compared to the control group .

Introduction

The body's non-specific, protective response to tissue damage is inflammation. Pathogens, abrasions, chemical irritants, cell deformation or disruption, and severe temperatures are some potential causes[1]. Rubor, calor, tumour, and pain are the four primary indicators of inflammation. Currently *functio laesa* (loss of function) is also considered as a symptom of inflammation. Inflammation can be broadly classified into acute and chronic. Initial and transient tissue reactions to damage occur during acute inflammation. Chronic inflammation causes successive, protracted tissue reactions following the initial reaction.[2]

Early applications of decoctions or formulations of specific plants and their extracts for the alleviation of pain, fever, and inflammation mark the beginning of the history of anti-inflammatory medications[3]. By 19th century salicylates were discovered out from the bark of Willow Spp., and Aspirin was created later. Non-steroidal anti-inflammatory medications

(NSAIDs) were first largely organic acids, but later non-acidic molecules were developed, thanks to the scientific breakthroughs of the 19th and 20th centuries. Nonsteroidal anti-inflammatory medications (NSAIDs) are a class of therapeutic pharmaceuticals that vary in their pharmacodynamics and structural characteristics but have a common method of action.[4]

Current era is in search of herbal medicines for meeting the health care needs. Of this Ayurveda is the science which have a strong scientific and historical ground. Validation of the local flora that are in folklore clinical practices can meet the increased need for herbal preparations to an extent. *Sebastiania chamaelea* (L.) Mull. Arg. of Euphorbiaceaea is one among them. The authentic reference for the drug was obtained from Hortus Malabaricus.[5] The pharmacological capability of the drug is well evident from the ayurvedic ancient texts like *Yogamrutham.*[6] After all preliminary pharmacological activity screening of the dug can bring out it into the mainline of treatment. With this thought the anti-inflammatory activity of the drug was assessed

Materials and Methods

A. *Materials*

1.Animal procurement

The animals were bought from College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala.

2.Collection and preparation of the test drug

The plant *Sebastiania chamaelea* (L). Mull.Arg. (Malayalam name – Kodiyaavanakku) was positively identified. In the month of November, the whole plant including the root, stem, leaves, flowers, fruits and seeds, were plucked up carefully. Only the matured drug was collected and the fruiting of the plant was considered as the indicator of maturity.

The drugs were gathered, thoroughly cleaned in water to remove physical contaminants, and then dried thoroughly in the shade. Properly dried drug was then made to a fine powder and passed through a sieve of 120 mesh size. This powder form of the drug was used for the study.

The powder suspension was made by combining 12 grammes of powder with 100 millilitres of distilled water (considering 12 gm. as the human dose). It was uniformly shaken so that 0.12 gramme of the test medication is present in 1 ml of the solution. This is given orally to the animals using feeding cannulas in accordance with their body weight.

3.Dose of the test drug

There was no available classical reference regarding the dose of *Sebastiania chamaelea* (L). Mull.Arg. The formula provided by M. N. Ghosh in Fundamentals of Experimental Pharmacology was used to determine the effective dose of the test medication for rats, which was determined to be 12 gm of powder equivalent to a human adult dose. On the basis of the body surface area ratio, the dosages of the drug were calculated by extrapolating the therapeutic dose to the rat dose (conversion factor 0.018 for rats)

Animal dose = 0.018 times the human dose for 200 g of animal:

= 12 gm x 0.018 = 0.216 gm / 200 gm of animal:

= 216 mg / 200 gm of animal

The medicine was administered in the following doses: (1/2)X, X, and 2X, where X stands for the test drug's estimated effective dose. (0.216 gm/200gm b. wt.)

Dose of Brewer's yeast:

Dose for 150 gm rat = 1.5 ml

Dose for 200 g rat = 2 ml.

4.Grouping of animals

The creatures were split into 4 groups of 6 rats each, including 3 males and 3 females. Group A (Control) was provided with a regular food and water. Treatment groups received the test drug in one of three doses: the predicted effective dose, half the calculated dose, and double the calculated dose.

Table No. 1: Grouping of animals

Groups	Drug Dose
A group (Control)	Standard Diet And Water
B group (Half Dose)	1/2 X (0.108gm/200gm body Wt.)
C group (Effective Dose)	X (0.216gm/200gm body Wt.)
D group (Double Dose)	2x (0.432gm/200gm body Wt.)

B. *Methods*

Animals were starved overnight without restricting water. A mark was put on the animal's left hind paw just above the tibia tarsal junction so that each time the paw was dipped in the mercury column of the plethysmograph up to a predetermined point, the paw volume would remain constant.

The animal was given 5ml of water using stomach tubes to achieve uniform hydration. Before the medication was given, the animal's initial left hind paw volume was noted. Thirty minutes after giving the water orally, 0.05 ml of formalin was subcutaneously administered into its left hind paw to cause acute inflammation. The study used an acute inflammation of paw volume rise of no less than 0.4 mm^3. The right paw was used as a standard for the healthy paw in comparison. The appropriate doses were then given to each group in turn. For four hours following medication administration, paw volume was recorded hourly. The oral route was used for a single administration for all groups.

Both within and between groups, the change in paw volume was compared. One-way ANOVA and Tukey's post hoc analysis were used to statistically analyze the data. Only after receiving approval from the institutional animal ethics committee was the study carried out.

Observations & Results

1.Comparison of Group A's paw volumes (control)

The mean paw volume before formalin injection in Group A (Control) was 0.2 ml which increased to 0.667 ml after formalin injection. Further the mean paw volume increased to 0.683 ml, 0.733 ml and 0.783 ml at 1st hr, 2nd hr and 3rd hr respectively. At 4th hr the mean volume remained constant like that of the 3rd hr.

Increased paw volume at 4th hr have shown statistical significance when compared to paw volume before formalin injection (BT), and this increase was not significant when compared to 1st hr, 2nd hr and 3rd hr. Also the paw volume at 3rd hr have shown significant increase when compared to BT. But this increase was not significant when compared to 1st hr, 2nd hr and 4th hr. All other changes in paw volume within the group were not statistically significant .

2.Comparison of Group B's paw volumes (Half dose)

The mean paw volume before formalin injection in Group B (Half dose) was 0.2 ml which increased to 0.667ml after formalin injection and remained the same even 1 hr after the medicine induction. Later it decreased to 0.550 ml, 0.500 ml and 0.433 ml at 2nd hr, 3rd hr and 4th hr respectively.

The decrease in paw volume at the 4th hr is significant when compared to the BT, 1st hr and 2nd hr, with high statistical significance at BT and 1st hr. But decrease is not significant on comparison with the 3rd hr. The decrease in paw volume at the 3rd hr is highly significant when compared to the BT and 1st hr, but it is not significant when compared to 2nd hr. Decreased paw volume at 2nd hr is significant when compared to 1st hr and BT. The difference in paw volume at 1st hr and BT was not statistically significant.

Table No. 2: Comparison of Group A's paw volumes (control)

Group A	Mean Difference	q	Significance	Summary	95% CI of diff
BT Vs 1st hr	-0.01667	0.6162	No	ns	-0.1330 to 0.09968
BT Vs 2nd hr	-0.06667	2.465	No	ns	-0.1830 to 0.04968
BT Vs 3rd hr	-0.1167	4.314	Yes	*	-0.2330 to -0.0003151
BT Vs 4th hr	-0.1167	4.314	Yes	*	-0.2330 to -0.0003151
1st hr Vs 2nd hr	-0.05000	1.849	No	ns	-0.1664 to 0.06635
1st hr Vs 3rd hr	-0.1000	3.697	No	ns	-0.2164 to 0.01635
1st hr Vs 4th hr	-0.1000	3.697	No	ns	-0.2164 to 0.01635
2nd hr Vs 3rd hr	-0.05000	1.849	No	ns	-0.1664 to 0.06635
2nd hr Vs 4th hr	-0.05000	1.849	No	ns	-0.1664 to 0.06635
3rd hr Vs 4th hr	0.0	0.0	No	ns	-0.1164 to 0.1164

Table No. 3: Comparison of Group B's paw volumes (Half dose)

Group B	Mean difference	q	Significance	Summary	95% CI of difference
BT Vs 1st hr	0.0	0.0	No	ns	-0.08546 to 0.08546
BT Vs 2nd hr	0.1200	6.124	Yes	**	0.03454 to 0.2055
BT Vs 3rd hr	0.1600	8.165	Yes	***	0.07454 to 0.2455
BT Vs 4th hr	0.2200	11.23	Yes	***	0.1345 to 0.3055
1st hr Vs 2nd hr	0.1200	6.124	Yes	**	0.03454 to 0.2055
1st hr Vs 3rd hr	0.1600	8.165	Yes	***	0.07454 to 0.2455
1st hr Vs 4th hr	0.2200	11.23	Yes	***	0.1345 to 0.3055
2nd hr Vs 3rd hr	0.04000	2.041	No	ns	-0.04546 to 0.1255
2nd hr Vs 4th hr	0.1000	5.103	Yes	*	0.01454 to 0.1855
3rd hr Vs 4th hr	0.0600	3.062	No	ns	-0.02546 to 0.1455

3.Comparison of Group C's paw volumes (Effective dose)

The mean paw volume before formalin injection in Group C (Effective dose) was 0.2 ml which increased to 0.683 ml after formalin injection and remained constant in the 1st hr after medicine induction. Then it decreased to 0.583 ml, 0.517 ml and 0.450 ml at 2nd hr, 3rd hr and 4th hr.

The decrease in paw volume at the 4th hr is statistically significant when compared to the BT, 1st hr. But it is not significant on comparison with the 2nd and 3rd hr. The decrease in paw volume at the 3rd hr is not significant when compared to BT, 1st and 2nd hr. Decreased paw volume at 2nd hr is not significant when compared to 1st hr and BT. The paw volume at 1st hr and BT shows no significant difference.

4.Comparison of Group D's paw volumes (Double dose)

The mean paw volume before formalin injection in Group D (Double dose) was 0.2 ml which increased to 0.667ml after formalin injection and remained same during the 1st hr after medicine induction. Then it decreased to 0.600 ml and 0.533 ml at 2nd hr and 3rd hr respectively. After 3rd hr it remained constant for the 4th hr.

The decrease in paw volume at the 4th hr is significant when compared to the BT and 1st hr. But it is not significant on comparison with the 2nd and 3rd hr. There is significant decrease in paw volume at 3rd hr when compared to BT and 1st hr. But this decrease is not significant when compared to the 2nd hr. Decreased paw volume at 2nd hr is not significant when compared to 1st hr and BT. The paw volume at 1st hr and BT shows no significant difference.

Table No. 4: Comparison of Group C's paw volumes (Effective dose)

Group C	Mean difference	q	Significance	Summary	95% CI of difference
BT Vs 1st hr	0.0	0.0	No	ns	-0.1722 to 0.1722
BT Vs 2nd hr	0.1000	2.499	No	ns	-0.07218 to 0.2722
BT Vs 3rd hr	0.1667	4.164	No	ns	-0.005513 to 0.3388
BT Vs 4th hr	0.2333	5.830	Yes	**	0.06115 to 0.4055
1st hr Vs 2nd hr	0.1000	2.499	No	ns	-0.07218 to 0.2722
1st hr Vs 3rd hr	0.1667	4.164	No	ns	-0.005513 to 0.3388
1st hr Vs 4th hr	0.2333	5.830	Yes	**	0.06115 to 0.4055
2nd hr Vs 3rd hr	0.06667	1.666	No	ns	-0.1055 to 0.2388
2nd hr Vs 4th hr	0.1333	3.331	No	ns	-0.03885 to 0.3055
3rd hr Vs 4th hr	0.06667	1.666	No	ns	-0.1055 to 0.2388

Table No. 5: Comparison of Group D's paw volumes (Double dose)

Group D	Mean difference	q	Significance	Summary	95% CI of difference
BT Vs 1st hr	0.0	0.0	No	ns	-0.1171 to 0.1171
BT Vs 2nd hr	0.06667	2.449	No	ns	-0.05042 to 0.1838
BT Vs 3rd hr	0.1333	4.899	Yes	*	0.01625 to 0.2504
BT Vs 4th hr	0.1333	4.899	Yes	*	0.01625 to 0.2504
1st hr Vs 2nd hr	0.06667	2.449	No	ns	-0.05042 to 0.1838
1st hr Vs 3rd hr	0.1333	4.899	Yes	*	0.01625 to 0.2504
1st hr Vs 4th hr	0.1333	4.899	Yes	*	0.01625 to 0.2504
2nd hr Vs 3rd hr	0.06667	2.449	No	ns	-0.05042 to 0.1838
2nd hr Vs 4th hr	0.06667	2.449	No	ns	-0.05042 to 0.1838
3rd hr Vs 4th hr	0.0	0.0	No	ns	-0.1171 to 0.1171

Table 6: Paw volume comparisons across groups one hour after medication administration

Groups	Mean Difference	q	Significance	Summary	95% CI of difference
Group A vs Group B	0.01667	0.5199	No	ns	-0.1102 to 0.1436
Group A vs Group C	0.0	0.0	No	ns	-0.1269 to 0.1269
Group A vs Group D	0.01667	0.5199	No	ns	-0.1102 to 0.1436
Group B vs Group C	-0.01667	0.5199	No	ns	-0.1436 to 0.1102
Group B vs Group D	0.0	0.0	No	ns	-0.1269 to 0.1269
Group C vs Group D	0.01667	0.5199	No	ns	-0.1102 to 0.1436

Table 7: Paw volume comparisons across groups two hour after medication administration

Groups	Mean Difference	q	Significance	Summary	95% CI of difference
Group A vs Group B	0.1833	5.880	Yes	**	0.05992 to 0.3067
Group A vs Group C	0.1500	4.811	Yes	*	0.02659 to 0.2734
Group A vs Group D	0.1333	4.276	Yes	*	0.009921 to 0.2567
Group B vs Group C	-0.03333	1.069	No	ns	-0.1567 to 0.09008
Group B vs Group D	-0.05000	1.604	No	ns	-0.1734 to 0.07341
Group C vs Group D	-0.01667	0.5345	No	ns	-0.1401 to 0.1067

Table 8: Paw volume comparisons across groups three hour after medication administration

Groups	Mean Difference	q	Significance	Summary	95% CI of difference
Group A vs Group B	0.2833	10.35	Yes	***	0.1749 to 0.3917
Group A vs Group C	0.2667	9.737	Yes	***	0.1583 to 0.3751
Group A vs Group D	0.2500	9.129	Yes	***	0.1416 to 0.3584
Group B vs Group C	-0.01667	0.6086	No	ns	-0.1251 to 0.09173
Group B vs Group D	-0.03333	1.217	No	ns	-0.1417 to 0.07506
Group C vs Group D	-0.01667	0.6086	No	ns	-0.1251 to 0.09173

Table 9: Paw volume comparisons across groups four hour after medication administration

Groups	Mean Difference	q	Significance	Summary	95% CI of difference
Group A vs Group B	0.3500	9.899	Yes	***	0.2101 to 0.4899
Group A vs Group C	0.3333	9.428	Yes	***	0.1934 to 0.4733
Group A vs Group D	0.2500	7.071	Yes	***	0.1101 to 0.3899
Group B vs Group C	-0.01667	0.4714	No	ns	-0.1566 to 0.1233
Group B vs Group D	-0.1000	2.828	No	ns	-0.2399 to 0.03994
Group C vs Group D	-0.08333	2.357	No	ns	-0.2233 to 0.05660

Discussion

The anti-inflammatory activity of the *choorna* of whole plant *Sebastiania chamaelea* (L.)Mull.Arg. in three different dose groups (half dose, effective dose and double dose) were assessed using the Formalin induced paw oedema method in Wistar albino rats. One way ANOVA with Tukey's post hoc analysis was employed to statistically analyze the data collected, and it was found that all three dose groups had statistically significant anti-inflammatory activity when compared to the control group at $p < 0.05$. By the second hour following the administration of the medication, all three groups had begun to exhibit an anti-inflammatory response, and by the fourth hour, the half-dose group had exhibited the greatest anti-inflammatory effect, with a p-value < 0.0001.

Different dose groups were chosen for understanding the effect of the drug on inflammation. In the study the control group doesn't show any decrease in the paw volume, instead it shows an increase in the volume. But in test dose groups the paw volume remains the same in the first hour which means the drug has the ability to block the progress of inflammation. By the second hour the drug have shown significant anti-inflammatory activity which means the drug have role in the repair mechanism. And the drug's anti-inflammatory impact is still noticeable after four hours, demonstrating the drug's long-lasting ability to reverse inflammation.

The drug contains phytochemical constituents like flavonoids, saponins, tannins, steroids and phenols. Flavonoids are known to have anti-inflammatory and antipyretic activities. And these all phyto constituents can be the reason behind the pharmacological action of the drug.

Conclusion

The detailed literary review regarding the drug among *Ayurvedic* as well as modern literatures has been done as a part of the study. The drug has been described in the scriptures including *Yogamrutam* and Hortus Malabaricus as *Kodiyaavanakku*. But the drug receives no special attention in the main stream of *Ayurveda* practices. Evidences are there for the use of this drug in Unani system of medicine. Rather than this, the drug was explored widely in folklore practice for treating diarrhoea, liver enlargement, leprosy and syphilis, for easy delivery, malaria, pain during dental flare up in infants and also as a nerve tonic.

From the study, it can be summarized that powder of the whole plant *Sebastiania chamaelea* (L.) Mull.Arg. possess significant anti-inflammatory activity.

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