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EXPERIMENTAL EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF *SAPTANGA GUGGULU* PREPARED BY *GUGGULU SHODHANA* WITH FOUR DIFFERENT MEDIA IN WISTAR STRAIN ALBINO RATS

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ABSTRACT

Introduction: *Saptanga Guggulu* is a well-known drug of Indigenous System of Medicine. *Guggulu* is a gummy resin of the plant. There are different media explained in literature for *Shodhana* of *Guggulu*. According to the media of purification the quality and pharmacological properties of *Guggulu* may vary. In the present analytical study four batches of *Guggulu* purified by four different media have been used to prepare four batches of *Saptanga Guggulu* and its anti-inflammatory experimental study was done **Methods**: *Guggulu Shodhana* was carried out as per the method mentioned in Ayurvedic Formulary of India. Four different batches of *Guggulu* were prepared by using four different media for the purification of *Guggulu* namely *Triphala Kwatha, Godugdha, Vasapatra Swarasa* and *Nirgundipatra Swarasa* along with *Haridra Churna*. Four batches of *Saptanga Guggulu* were prepared with *Guggulu* purified by each of the *Shodhana* media as per the reference mentioned in *Bhaishajya Ratnavali, Vranashotha Adhikara* and its anti-inflammatory experimental study was done. The batches were names as Test drug I - *Saptanga Guggulu* prepared by *Triphala Kwatha Shodhita Guggulu*, Test drug II - *Saptanga Guggulu* prepared by *Godugdha Shodhita Guggulu*, Test drug III - *Saptanga Guggulu* prepared by *Vasapatra Swarasa Shodhita Guggulu* and Test drug IV - *Saptanga Guggulu* prepared by *Nirgundipatra Swarasa and Haridra Churna Shodhita Guggulu*. **Results:** The results obtained from the experimental study can be used as a standard to determine the potent anti-inflammatory effect of *Saptanga Guggulu*. The various media used for the purification of *Guggulu* can also be standardised by the media of purification which provides the best anti-inflammatory action. **Conclusion:** All four batches of *Saptanga Guggulu* showed varied effects in Carrageenan induced hind paw oedema for acute inflammation. All the four batches produced weak anti-inflammatory effect in cotton pellet implanted granuloma model for chronic inflammation.

Keywords: *Saptanga guggulu*, Experimental Study, Acute-inflammation, Chronic-inflammation.

INTRODUCTION

Guggulu[1] is a well-known drug since the Vedic period. The drug *Saptanga Guggulu*[2] is a purely herbal drug which has been mentioned in the textbook of *Bhaishajya Ratnavali*, in the *Vranashotha chikitsa* chapter. It contains seven herbs followed by *Guggulu*. All these combined together is suggested to have anti-inflammatory action. There are many available drugs in the market which exhibit anti-inflammatory action. Among the many formulations mentioned in *Ayurveda*, *Saptanga Guggulu* is not available in the market. So in the present study this has been taken to evaluate its anti-inflammatory effect. The drug will be tested on Wistar strain albino rats for its effect in both acute as well as chronic inflammation. *Guggulu* as a raw drug cannot be administered directly in the body for achieving therapeutic effect. It contains many impurities. So it has to undergo the process of purification. There are many methods mentioned in classics for the purification of *Guggulu*[3]. In the present study the method used for purification of *Guggulu* is *Swedana*. For this process various liquid media have been mentioned in classics. Here in the present study four media have been selected namely *Triphala kwatha, Godugdha, Vasapatra swarasa* and *Nirgundipatra swasasa* along with *Haridra churna*. Four batches of *Guggulu* will be tied in a poultice and suspended in these four media and heated for 3 hours continuously. By this the therapeutically effective *Guggulu* will dissolve in the media. The remaining *Guggulu* along with the impurities will be discarded. The media is then heated until it attains further thicker consistency. It is then dried and used for therapeutic purpose. Inflammation is a response of a tissue to injury or infection, and is necessary for normal repair and healing.

Acute inflammation is the result of rapid and complex interplay between the cells and soluble molecules of the innate immune system. The classical external signs include heat, redness, pain and swelling (calor, rubor, dolor, oedema). The inflammatory process is initiated by local tissue injury or infection. Damaged epithelial cells produce cytokines and antimicrobial peptides, causing early infiltration of phagocytic cells. As a result, there is production of leukotrienes, prostaglandins, histamine, kinins, anaphylotoxins and inducible nitric oxide synthase within inflamed tissue. The effect is vasodilatation and increased local vascular permeability. Failure to remove an inflammatory stimulus results in chronic inflammation. Persisting microorganisms stimulate the ongoing accumulation of neutrophils, macrophages and activated T-lymphocytes. If this is associated with local deposition of fibrous connective tissue, a granuloma may form. This is characteristic of infections such as tuberculosis and leprosy, in which the microorganism is protected by a cell wall which shields it from killing despite phagocytosis[4]. The disease *Shotha* is explained in *Charaka Samhita*[5,6] *Sushrutha Samhita*[7,8], *Ashtanga Samgraha*[9,10], *Ashtanga Hridaya*[11,12], *BhavaPrakash*[13], *Madhava Nidana*[14] and many others. The signs and symptoms of *Shotha* can be compared with the signs of inflammation such as *Davathu* (dolor/pain), *Sira ayama* (dilatation of veins), *Siratanutva* (increased vascular permeability), *Ushma* (calor/heat), *Angavivarnata* (erythema or discoloration of the affected site), *Gaurava* (heaviness due to exudation of protein rich fluid into extravascular spaces), *Utsedha*[15] (tumor/swelling).

The drug *Saptanga Guggulu* has been attributed with *Shothahara* property according to *Bhaishajya Ratnavali.* With this perspective, the four batches of *Saptanga Guggulu* prepared by four batches of *Guggulu* purified by four different media (*Triphala Kwatha, Godugdha, Vasapatra Swarasa, Nirgundipatra Swarasa* with *Haridra Churna*) were comparatively studied with respect to *Shothahara* (anti-inflammatory) activity in animal models.

Methods

Guggulu Shodhana

Guggulu Shodhana was carried out as per the method mentioned in Ayurvedic Formulary of India. Four different batches of *Guggulu* were prepared by using four different media for the purification of

Guggulu namely *Triphala Kwatha, Godugdha, Vasapatra Swarasa* and *Nirgundipatra Swarasa* along with *Haridra Churna*.

Preparation of *Saptanga Guggulu*

Saptanga Guggulu was prepared as per the reference mentioned in *Bhaishajya Ratnavali, Vranashotha Adhikara*.

Ingredients of the drug *Saptanga Guggulu*.

The drug *Saptanga Guggulu* taken for the present study has been taken from *Bhaishajya Ratnavali, Vranashotha Adhikara*. The ingredients and ratio of the quantity of the drugs are:

Table 1: Showing the ingredients and the ratio of the quantity of the drugs

Sanskrit name	Botanical name	Family	Part used	Quantity
1.*Vidanga*	*Embilica ribes* Burm. F	Myrsinaceae	Fruit	1 part
2.*Haritaki*	*Terminalia chebula* Retz.	Combrataceae	Fruit	1 part
3.*Vibhitaki*	*Terminalia bellerica* Roxb.	Combrataceae	Fruit	1 part
4.*Amalaki*	*Embilica officinalis* Gaertn.	Euphorbiaceae	Fruit	1 part
5.*Shunti*	*Zingiber officinale* Roscoe.	Zingiberaceae	Rhizome	1 part
6.*Maricha*	*Piper nigrum* Linn.	Piperaceae	Fruit	1 part
7.*Pippali*	*Piper longum* Linn.	Piperaceae	Fruit	1 part
8.*Guggulu*	*Commiphora mukul* (Hook ex. Stocks) Engl.	Burseraceae	Gum	7 parts

Collection of the drugs

All the ingredients required for the preparation of the drug were collected from Alva's Pharmacy, Mijar, Moodabidri and certified by the botanist and pharmacognosy experts.

The fine powder of the ingredients 1 to 7 (Shown in Table 1) were taken in the quantity one part each and seven parts of *Guggulu* was taken. *Guggulu* was added to the *Khalva Yantra* and the fine powder of the other ingredients was added little by little in quantity and triturated until uniform mixtures of the ingredients were obtained. It was then rolled into pills of suitable size by applying ghee in the palm of the hands. It was then dried and used for the study.

Chemical material

a. Carrageenan was obtained from HiMedia Laboratories Pvt. Limited, Mumbai bearing the batch number RM 1576-100 G.
b. Carboxymethl cellulose (CMC) bearing the batch number QF1Q610371 was obtained from Merck Specialities Private Limited, Mumbai.

Experimental animals

Albino rats of Wistar strains of either sex between 150 to 250 g were obtained from animal house attached to department of Pharmacology, SDM Research Centre, Udupi. The experimental protocol was approved by the institutional ethical committee under the reference no. SDMCAU/IAEC/MB/RS/05. The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided had the following conditions: controlled lighting of 12:12h light and dark cycle, temperature of 25ºC and relative humidity of approximately 50%.

Dose fixation for the animals

The dose fixation for experimental animals was done referring to the standard table of Paget and Barnes (1964) formula involving body surface area ratio.

Test groups

The human dose for *Vati* is one *Karsha* i.e. 12 g as mentioned in *Sharangdhara Samhita, Madhyama Khanda. Vati Kalpana*[16]. But the dose taken here was 1 g as the drug is being tested for the first time.

Dose for rats = Therapeutic Human dose × 0.018 × 5 g/kg

= 1 x 0.018 x 5 g/kg

= 0.09 g/kg

= 90 mg/kg

Standard group for Carrageenan induced paw edema test

The standard drug selected for the study was tablet Diclofenac sodium of 50mg. A homogeneous solution of the drug was prepared by adding 50mg of CMC (Carboxymethyl cellulose) and 10 ml of distilled water to it. The standard dose of tablet Diclofenac sodium for humans is 25mg/kg. Therefore, for one gram it is 0.025mg.

Dose for rats = 0.025mg X body weight of individual rat

The obtained value of the dosage is expressed in terms of mg.

Standard group for Cotton pellet implanted granuloma test

The standard drug selected for the study was tablet Diclofenac sodium of 50mg. A homogeneous solution of the drug was prepared by adding 50mg of CMC (Carboxymethyl cellulose) and 10 ml of distilled water to it. The standard dose of tablet Diclofenac sodium for humans is 25mg/kg. Therefore, for one gram it is 0.025mg.

Dose for rats = 0.025mg X body weight of individual rat

The obtained value of the dosage is expressed in terms of mg.

Animal grouping

Adult Wistar albino rats of either sex weighing 150 to 250 g were divided into six different groups comprising of six rats in each group. Control group animals were administered with normal tap water at a dose of 5ml /kg in 0.5% gum acacia, animals of standard group were administered with Diclofenac sodium at a dose of 25 mg/kg for acute anti-inflammatory activity and also for chronic anti-inflammatory activity. The test groups were administered with the test drugs at a dose of 90 mg/kg for rats.

Route of administration

The drugs were administered orally by using rat feeding tube fixed to syringe.

Table 2: Showing the grouping of animals for both study models

Group	No:of rats	Drug	Form	Purpose
Control	6	Water	Nil	To observe the changes occurring in the swelling and also to compare the anti-inflammatory effect of the other group.
Standard	6	Diclofenac sodium		To assess the anti-inflammatory effect.
Test drug I	6	*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Triphala Kwatha*.		To assess the anti-inflammatory effect.
Test drug II	6	*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Godugdha*.		To assess the anti-inflammatory effect.
Test drug III	6	*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Vasapatra Swarasa*.		To assess the anti-inflammatory effect.
Test drug IV	6	*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Nirgundipatra Swarasa* with *Haridra Curna*.		To assess the anti-inflammatory effect.

Evaluation of anti –inflammatory activity

a. Acute anti - inflammatory activity.

The acute anti-inflammatory activity was evaluated by carrageenan induced hind paw oedema test in Wistar albino rats by method of Winter *et al*.[17] The group specific drugs were administered orally for seven consecutive days and 1hour before the carrageenan injection on 7th day. Acute inflammation

was produced by injecting 0.1 ml of 1% carrageenan solution into sub plantar surface of rat's hind paw. The paw volume up to the tibio-tarsal articulation was measured using a Plethysmometer (PLM-01 PLUS Orchid Scientifics) at basal, 1st hour, 3rd hour, 6th hour and 24th hour after carrageenan injection.

The anti-inflammatory activities were expressed as percentage decrease in paw oedema using the following formula:

$$\% \text{ change in paw oedema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c is the paw volume of control group and V_t is the paw volume of test group.

b. Chronic anti-inflammatory activity

The effect of test drug on cotton pellet implanted granuloma formation in rats was studied as per the method described by D'arcy *et al* (1960). The rats were anaesthetised under ketamine (80 mg/kg, i.p). The dorsum was shaved and swabbed with 70% (v/v) alcohol. Mid-line incision of 1cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of small blunt forceps. One sterile cotton pellet weighing 100 mg (prepared by rolling of a cotton piece of 100 mg and sterilised by autoclaving for 30 min under 15 lbs pressure) was inserted per tunnel and the incision was closed with interrupted sutures after expelling the air from the tunnel. Group specific drugs were administered for seven consecutive days starting from the day of implantation. The rats were sacrificed on 8th day and dissected for collection of lymph node, spleen, adrenal glands. Implanted cotton pellets were removed and cleaned of extraneous tissues and dried by placing them in a hot air oven overnight at 80°C and then weighed. The difference between the initial weight and the final weight of the pellet after drying was taken as the weight of granuloma tissue. The result was expressed as mg of granulation tissue formed per 100 g body weight. The weight of spleen, adrenal gland and lymph node were noted. The dissected organs were preserved in 10% formalin and sent for histopathological examination. In addition, blood was withdrawn from retro-orbital plexus with the help of capillary tubes and the samples were collected to estimate biochemical parameters.

Result

Statistical analysis

The experimental data were expressed as mean ± SEM. The data obtained was analysed by using one way analysis of variance (ANNOVA) followed by Dunnett's't' test for determining the level of significance of the observed effects. A 'p' value of less than 0.05 was considered statistically significant. Graph Pad In Stat-3 was used for statistical analysis of the generated data.

a. Acute inflammation

Carrageenan induced paw oedema

Table 3: The effect of test drug on percentage increase of paw oedema at different time interval

		Mean±SEM			
		Paw volume recorded at different time intervals (ml)			
Group	**Dose mg/kg**	1st hour	3rd hour	6th hour	24th hour
Control(normal tap water)	5 mL/kg	1.25±0.08	1.66±0.03	1.78±0.11	1.07±0.04
Standard drug	25 mg/kg	1.03±0.04	1.05±0.06	0.99±0.00	0.93±0.02
Test drug I	90 mg/kg	1.31±0.05	0.21±0.07	1.33±0.06	0.99±0.05
Test drug II	90 mg/kg	1.29±0.07	1.40±0.06	1.16±0.07	1.03±0.05
Test drug III	90 mg/kg	1.07±0.06	1.23±0.08	1.15±0.05	0.85±0.02
Test drug IV	90 mg/kg	0.96±0.05	1.10±0.09	1.10±0.04	0.88±0.04

Data: MEAN ± SEM.

Data related to the effect of test drugs on paw oedema of carrageenan induced paw oedema at different time intervals is shown in the table 3.

Table 4: The effect of test drug on percentage inhibition of paw volume at different time interval

		Mean±SEM Percentage increase of paw oedema at different time interval.			
Group	**Dose mg/kg**	1st hour	3rd hour	6th hour	24th hour
Standard drug	25 mg/kg	32.77↓	67.90↓**	79.13↓**	25.35↓
Test drug I	90 mg/kg	32.88↑	49.07↓*	47.99↓**	8.95↓
Test drug II	90 mg/kg	7.96↑	38.74↓	72.56↓**	0.85↑
Test drug III	90 mg/kg	17.44↑	23.04↓	43.75↓**	25.35↓
Test drug IV	90 mg/kg	29.02↓	45.69↓*	66.06↓**	13.22↓

Data: MEAN ± SEM, **p<0.01 in comparison to control group,* p <0.05 in comparison to control group.

Data related to the effect of test drugs on percentage increase of carrageenan induced paw oedema is shown in the table 4.

b. Chronic inflammation

Cotton pellet implanted granuloma

Table 5: Effect of test drugs on cotton pellet implanted granuloma formation

Group	**Dose mg/kg**	**Dosage form**	**Mean±SEM Granuloma tissue weight (mg)/100gbody weight**	% change
Control(normal tap water)	5 mL/kg	Liquid	151.95±18.92	-
Standard drug	25 mg/kg	Liquid	109.51±16.12	27.93↓
Test drug I	90 mg/kg	Liquid	122.23±13.93	19.55↓
Test drug II	90 mg/kg	Liquid	172.83±36.22	13.74↑
Test drug III	90 mg/kg	Liquid	261.13±45.43*	71.85↑
Test drug IV	90 mg/kg	Liquid	162.92±8.23	7.21↑

Data: MEAN ± SEM, * p <0.05 in comparison to control group

Data related to the effect of test drug on weight of cotton pellet has been shown in the table 5.

DISCUSSION

Experimental study

For any drug to be established in the clinical trial, it becomes a need to evaluate its safety and efficacy in preclinical studies in the form of *in vitro* and *in vivo* animal experiments. Thus, selection of an animal model is one of the most important steps in any of the experimental pharmacological study. Animals are phylogenetically closer to man. They produce similar disease profile as in humans due to similarity in anatomy, physiology and biochemistry.

The four drugs (*Saptanga Guggulu* prepared by the different methods of *Guggulu Shodhana*) were evaluated for anti-inflammatory activity employing two experimental models representing acute and chronic inflammatory reactions.

Acute inflammation

Carrageenan induced hind paw oedema test is based on the principle of release of various inflammatory mediators by carrageenan. The test is selected because of its sensitivity in evaluating orally active anti-inflammatory drugs particularly in acute phase of inflammation. Oedema formation due to carrageenan in the rat paw is biphasic, where in the first phase begins immediately after injection of carrageenan with the release of histamine, serotonin and kinins from local tissue damage and act on the vascular permeability lasting up to two hours. The second phase begins at the end of first phase and is due

to the release of prostaglandins, protease and lysosomes by tissue macrophages after 3rd hour of tissue damage and lasts for a duration of three to six hours. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasation, increased tissue water and plasma protein exudation, along with neutrophil extravasation, due to the metabolism of arachidonic acid.

From the critical analysis of the results, it was observed that standard drug has a highly significant anti-inflammatory activity at the 3rd hour and both test drug I and IV have a significant anti-inflammatory activity during the 3rd hour of inflammation i.e. early phase of inflammatory process. This might be due to inhibition of early release of histamines, serotonins and prostaglandins from the damaged tissue. Both the test drug II and III have shown mild to moderate activity during 3rd hour of inflammation which indicates their reduced potency to inhibit the release of histamine, serotonin and kinins from local tissue damage and act on the vascular permeability. The anti-inflammatory activity gradation at the 3rd hour after Carrageenan injection is – Test 1< Test 4< Test2 < Test 3. Test 1 and test 4 exhibited almost equal potency.

The standard drug as well as all the test drug groups showed a highly significant anti-inflammatory activity during the 6th hour of inflammation i.e. the second phase of inflammatory process which indicates their high potency in inhibiting the release of inflammatory mediators such as prostaglandins and leukotrienes from tissue macrophages. The observed effect was moderate to significant in magnitude. The anti-inflammatory activity gradation at the 6th hour after Carrageenan injection is – Test 2< Test 4< Test 1 < Test 3. In addition attempt was made to grade the observed activity taking into consideration the observed activity at all the three time intervals including whether inflammatory suppression was observed at all the time intervals. Based on this the best anti-inflammatory activity profile – the test 4 group exhibited best activity profile by suppressing paw oedema at all-time intervals , almost in similar fashion to the standard drug. The next best profile was observed in test 2 group in which marginal effect was observed at the initial period, moderate effect in the middle and very good suppression at the 6th hour. *Saptanga guggulu* prepared by *Guggulu Shodhana* with *Nirgundipatra Swarasa* along with *Haridra Churna* thus exhibits good activity profile and the next best effect with *Saptanga guggulu* prepared by *Guggulu Shodhana* with *Godugdha*. This clearly provides evidence for the role of different drug processing methods in ensuring potentiation of pharmacological activity.

Chronic inflammation

Cotton pellet implanted granuloma model is based on the foreign body granuloma that can provoke by subcutaneous implantation of pellets of compressed cotton in rats. The cotton pellet granuloma method has been widely used to assess the 3 phases of chronic inflammation, which are 1) a transudative phase, defined as the increase in the wet weight of the pellet that occurs during the first 3 hours, 2) an exudative phase, defined as plasma leaking from the blood stream around the granuloma that occurs between 3 hours and 72 hours after the implantation of pellet and 3) a proliferative phase, measured as the increase in the dry weight of the granuloma that occurs between 3 and 6 days after the implantation and thus to evaluate the anti-proliferative effect of drugs. The cotton pellets implanted in the intrascapular region induces a chronic inflammation process in which development of proliferative cells, monocyte migration, liquid accumulation, apoptosis, damage and so on will occur in the surrounding tissue of the pellets and these accumulations will produce a granulation tissue that covers the pellets. The anti-proliferative effect of anti-inflammatory drugs is by the prevention of collagen fibre formation and suppression of mucopolysaccharides. From the critical analysis of results, the standard drug as well as the test drug one has shown a slight decrease in the granulomatous tissue compared to that of the control group. The observed effect was found to be statisti-

cally non-significant. This is surprising since the standard drug was supposed to suppress granulation tissue formation in a significant manner. The reasons are not known- possibly due to dosage related aspects. The test drug III group showed a highly significant increase in the granulomatous tissue in comparison to that of the control group which indicates it weak potency. The test drug II and IV groups have shown a mild increase in the granulomatous tissue in comparison to the control group. Thus, these samples seem to lack anti-inflammatory effect in this model. The reasons are not known – possibly due to the dosage related aspects.

All the groups showed a mild to moderate decrease in the weight of the spleen compared to that of the control group which was statistically non-significant. But the test drug IV administered group showed more decrease in spleen weight compared to the other groups which indicates the hyper activity of the organ or decreased cellularity. Histological examination shows increase in the white pulp proportion. This is indicative stimulation of the activity.

The test drug I administered group showed an increase in the weight of the lymph node when compared to that of the control group. All the other groups showed a decrease in the weight of the lymph node, however the changes were found to be statistically non-significant. Histological examination showed inconsistent profile in lymph node.

All the groups except standard group showed an increase in the weight of the adrenal gland compared to the control group which was found to be statistically non-significant. The test drug III administered group showed more increase in the weight of the spleen compared to the other groups which was also found to be statistically non-significant. Histological examination revealed increase in the cellularity in Z reticularis. This zone is involved mainly in the formation of androgens mainly dehydroepiandrosterone (DHEA) and DHEA-sulfate. This may contribute to some extent to the observed anti-inflammatory activity.

Haemogram:

Moderate to significant decrease in total WBC count was observed in test drug administered group especially in group III and IV. This decrease in comparison to the inflammatory control may be indicative of suppression of inflammation. However, it did not correlate with observed activity in the cotton pellet granuloma model. The individual components were not affected to significant extent. Significant decrease was observed in RBC count in standard drug administered group. This may be due to RBC lysis. Moderate effect was observed with test drug –II – indicating it may share similar effect. The changes in other indices reflected this trend. The standard drug administered group showed a significant decrease in the Hb% compared to the control group which is indicative of the Hb% reducing property of NSAID. Test drug I and III administered groups showed a mild increase in the Hb% which was found to be statistically non-significant. The test drug II and IV administered groups showed a mild to moderate decrease in the Hb%, which was also found to be statistically non-significant. All the groups showed an increase in the neutrophil count compared to that of the control group. However the changes were found to be statistically non-significant. There was a mild to moderate decrease in the lymphocyte count observed among all the groups compared to the standard group, which was found to be statistically non-significant. The test drug I and II administered group showed a mild to moderate decrease, whereas all the other groups showed an increase in the monocyte count in comparison to the control group respectively. The changes were observed to be statistically non-significant. Test drug IV administered group showed a very significant decrease in the MCHC when compared to that of the control group. The test drug III administered group showed a significant increase in the MCHC when compared to that of the control group. The standard drug, test drug I and test drug II administered groups also showed an increase in the MCHC levels but however it was found to be statistically non-significant when compared to the control group. Significant increase in platelet count

was observed in standard and test II drug- This may be reactive thrombocytosis due to loss of blood as indicated by decrease in RBC count. The standard drug and the test drug II administered group showed a very significant increase in the platelet count when compared to that of the control group. The test drug I and III groups showed an increase in the platelet count when compared to the control group, but the data obtained did not attain statistically significant level. The test drug IV administered group showed a decrease in the platelet count and it was found to be statistically non-significant when compared to that of the control group. Serum C-reactive protein (CRP) is a specific abnormal protein that appears in the blood in response to inflammatory cytokines such as interleukin-6 (IL-6) during an inflammatory process. It is one of the most sensitive acute – phase reactants. CRP levels in the body have been used as a marker or indicator of inflammation. From the critical analysis of the results, the test drug II administered group did not show any change in the CRP level compared to the control group. All the other groups showed a mild to moderate increase in the CRP levels, which were found to be statistically non-significant.

The changes do not correlate with the observed effect on cotton granuloma formation. If the drug had good anti-inflammatory effect in this model representing proliferative phase of chronic inflammation this should have resulted in significant decrease in the CRP level- instead a mild increase was observed. This may be reflective of absence of significant anti-inflammatory effect against chronic inflammation.

Analysis of the results indicates presence of moderate to good anti-inflammatory activity in the four test groups with best activity in group -4. However, this type of activity was not observed on chronic inflammation.

Probable mode of action of the drug

The probable mode of action of the drug *Saptanga Guggulu* can be justified by the chemical composition or the secondary metabolites present in each ingredients of the drug.

By analysing the chemical constituents present in the ingredients of *Saptanga Guggulu* it is evident that the di-isobutyl amino derivatives present in *Vidanga* (*Embilica ribes* Burm. F) has anti-inflammatory effect[18]. The flavonoids present in *Haritaki* (*Terminalia chebula* Retz.) are known to inhibit prostaglandin synthesis. In addition to flavonoids, tannins are also known to possess analgesic activity. Since prostaglandins involved in pain perception are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids and tannins present in *Haritaki* (*Terminalia chebula* Retz.) is responsible for its analgesic and anti-inflammatory effect[19]. The gallic acid, chebulagic acid and corilagin present in *Vibhitaki* (*Terminalia bellerica* Roxb.) are responsible for the anti-inflammatory activity of the drug. They produce their effect by inhibition of the inflammatory pathway[20]. The phytochemicals like embilicanins A and B, gallic acid and ellagic acid present in *Amalaki* (*Embilica officinalis* Gaertn.) are powerful free radical scavengers. Moreover geraniin, carilagin, furosin present in the drug have nitric oxide scavenging property. The L-ascorbic acid present in *Amalaki* (*Embilica officinalis* Gaertn.) is a well-established anti-oxidant[21]. The gingerol present in *Shunti* (*Zingiber officinale* Roscoe.) is the major active component. They inhibit the prostaglandin and leukotriene biosynthesis which occurs during the inflammatory process[22]. Piperine which is the major active component of both *Maricha* (*Piper nigrum* Linn.) and *Pippali* (*Piper longum* Linn.) helps in inhibiting the production of the two important pro-inflammatory mediators IL6 and PGE_2[23]. Myrrhanol which is a triterpenoid present *Guggulu* (*Commiphora mukul* (Hook ex. Stocks) Engl.) has a potent anti-inflammatory effect[24]. This is how the drug *Saptanga guggulu* exerts it anti-inflammatory effect.

Limitations of the present study

In the present study as the drug was tested for the first time, the maximum dosage was fixed at 1 gram. So results were not clearly evident. Increased dosage

administration of the drug *Saptanga Guggulu* may produce even better results.

Scope for further study

- Since the maximum dosage which can be administered as per classics i.e. 12 g. is not taken here, further animal studies can be conducted by increasing the dosage of the drug and observing the effect.
- Other models of acute and chronic inflammation could be tested.
- The effect of the drug *Saptanga Guggulu* in diseases other than *Shotha* mentioned in classics can be carried out.
- Clinical trials can be undertaken to assess the therapeutic effect in humans.

CONCLUSION

The *shothahara karma* attributed to the drug is substantiated by *Katu* and *Tikta Rasa*, *Laghu* and *Ruksha Guna*, *Ushna Veerya* and *Vata – Kapha Dosha Hara* properties. The chemical constituents identified in the ingredients of *Saptanga Guggulu* have potent anti-inflammatory effect either individually or synergistically.

In Carrageenan induced hind paw oedema for acute inflammation, test drug I (*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Triphala Kwatha*) and test drug IV (*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Nirgundipatra Swarasa* with *Haridra Curna*) have shown significant suppression in oedema at the third hour of inspection, whereas test drug II (*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Godugdha*) and test drug III (*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Vasapatra Swarasa*) produced mild to moderate effect. All the four test drugs showed a very significant anti-inflammatory activity during the sixth hour of inspection. Test drug IV (*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Nirgundipatra Swarasa* with *Haridra Curna*) has the best anti-inflammatory activity followed by Test drug II (*Saptanga guggulu* prepared by *Guggulu Shodhana* with *Godugdha*). This clearly provides evidence for the role of different drug processing methods in ensuring potentiation of pharmacological activity.

All the test drugs have produced weak anti-inflammatory effect in cotton pellet implanted granuloma model for chronic inflammation.

Hence there is a significant effect of *Saptanga guggulu* prepared by either of the methods of *Guggulu Shodhana* in *Shotha*.

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List of figures

Histopathology slides

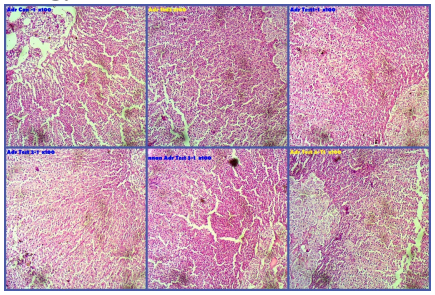

Figure 1: Showing the micrograph of adrenal gland of all six groups

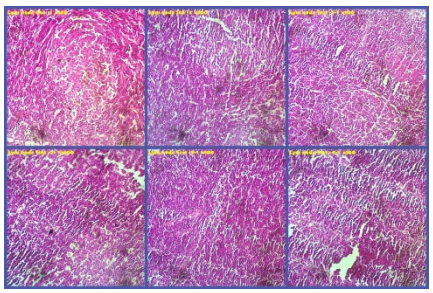

Figure 2: Showing the micrograph of lymph node of all the six groups

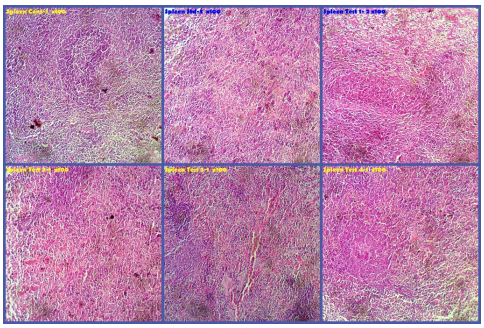

Figure 3: Showing the micrograph of spleen of all the six groups

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