



Research Article

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EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF TRAYODASHANG GUGGULU: AN AYURVEDIC FORMULATION IN COMPARISON WITH ALLOPATHIC DRUGS

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ABSTRACT

The main objective was to assess the *in vitro* anti inflammatory activity of the Ayurvedic formulation Trayodashang Guggulu in comparison to commercially available allopathic drugs. Anti inflammatory activity was estimated by inhibition of protein denaturation method, which in turn was preceded by adapting two different types of proteins which are egg albumin and bovine serum albumin. These were incubated along with actives and incubated in carefully controlled experimental conditions. The Ayurvedic formulation that is prepared prior was compared with six commercially available allopathic standards. The study results showed inhibition of protein according to the concentration of the active used. The Ayurvedic formulation showed prominent anti inflammatory activity better than the allopathic drugs at all concentrations that are selected between 200 to 1000µg/ml except when compared to Diclofenac. This study showed that the Ayurvedic combination formulation showed good anti inflammatory properties when tested for extent of protein denaturation. But the effect due to each of its constituents should be evaluated further.

Keywords: Anti inflammatory, Trayodashang Guggulu, Allopathic drugs, Protein denaturation.

INTRODUCTION

The groups of drugs that are specifically for the treatment which reduces inflammatory reactions are called anti-inflammatory drugs. Half of the analgesics are anti-inflammatory drugs, which act by reducing swelling, unlike the opioids which act on central nervous system by blocking the pain signaling mechanism of brain. Nonsteroidal anti-inflammatory drugs (NSAIDs) attenuate soreness by impeding the cyclooxygenase (COX) enzyme. Since, prostaglandins are synthesized from COX enzymes, which in turn cause inflammation. In whole, the NSAIDs prevent the prostaglandins from ever being synthesized, reducing or eliminating the pain. A few commonly used NSAIDs are Aspirin, Ibuprofen, and Diclofenac etc¹. The newer selective COX inhibitors are not categorized along with the older, traditional NSAIDs. The newer specific COX-inhibitors are not classified together with the traditional NSAIDs despite the fact that they presumptively have the similar mechanism of action².

On the contrary, there are some NSAIDs, which have good analgesic properties, but have very minimal anti inflammatory effect. For instance, paracetamol has a greater pain reducing properties than inflammation properties. Inflammation, pain erythema is caused as a result of human body's natural defense mechanism against injuries. To relieve from these effects, NSAIDs play a effective role by various inflammatory pathways. But at the same time they can cause a broad category of side effects which are very much undesirable such as gastric ulceration, stroke, myocardial infarction and sometimes toxicity at varied levels³.

On the other hand, most of the herbal formulations or the herbal drugs possess similar effectiveness while treating both acute as

well as chronic inflammation and pain syndromes with almost none or very low side effects. So to say because of the above mentioned reasons and due to consideration of the side effect profiles of the allopathic anti inflammatory drugs these days, there has been a greater interest in usage of herbal remedies and natural compounds⁴. In fact, many of the currently preferred natural compounds work by the same mechanism by inhibiting the inflammatory pathway such as NSAIDs⁵.

In this work we established the fact of how much better are the commonly used allopathic anti inflammatory drugs to a herbal Ayurvedic formulation which is potentially known to be a good anti inflammatory remedy by a *in-vitro* anti inflammatory test. This is to prove the efficacy difference between the herbal and allopathic formulations.

Trayodashang Guggulu (also spelled as Trayodashang Guggulu) is Guggulu based herbal formulation. It aids in strengthening of ligaments, muscles, bones and joints. It is efficient in problems related to the musculoskeletal and nervous system. Along with Gokshurad Guggulu and Ashwagandha powder in combination, it is known to have greater effect against osteoarthritis. It is advantageous in all kinds of pain such as gout, paralysis, sciatic pain, hemiplegia, joint pain, etc. it is proved to be working well when pain and weakness are associated side by side.

Therapeutic Indications

Trayodashang Guggulu is helpful in following health conditions.

- Brain & Nerves: Paralysis, Facial paralysis, Hemiplegia, Sciatica.
- Pain of neurological origin: Muscle, Bones & Joints, Osteoarthritis

- Bone pain: Osteoporosis or low bone mineral density
- Muscle spasm: Backache

Trayodashang Guggulu Benefits & Uses

The primary action of Trayodashang Guggulu is on joints, bones, nerves and muscles. For this reason it plays a major role in treatment of all the above stated conditions. Here are some primary benefits and medicinal applications of Trayodashang Guggulu.

- Paralytic disorders (facial paralysis or hemiplegia)
According to an ancient Ayurvedic literature, the main indication of Trayodashang Guggulu is its use in paralytic disorders. It works well when you start the medication in the first month of paralytic attack. Sometimes it helps even in the chronic cases, but then its outcome is low.
- Osteoarthritis and Osteoporosis (low bone mineral density)
It helps in cessation of wear and tear of joints and cartilages in case of osteoarthritis. However, in case of osteoporosis Trayodashang Guggulu do not increase bone mineral density directly, so we require supplements of calcium, zinc, magnesium and vitamin D. It prevents from fractures and bone injuries by giving strength to bones.

Trayodashang Guggulu Dosage

The nominal dose of Trayodashang Guggulu starts from 750mg and not more than 1.5grams twice a day. Although a dose less than this is not much effective in osteoarthritis and paralytic disorders, however, a combination of mineral supplements and Lakshadi Guggulu can be used as supportive therapy. Loss of appetite, Belching, Stomach upset and Indigestion is a few side effects that are observed only with high dosage that is more than 6 grams per day⁶.

Table 1: Plant Material- Trayodashang Guggulu Ingredients

Ayurvedic medicine	Botanical name	Quantity
Abha	<i>Acacia nilotica</i> Linn. / <i>Indica</i> / <i>Arabica</i> Willd	1 part
Ashwagandha	<i>Withania somnifera</i>	1 part
Hapusha	<i>Juniperus communis</i> Linn.	1 part
Guduchi	<i>Tinospora cordifolia</i>	1 part
Shatavari	<i>Asparagus racemosus</i>	1 part
Gokshura	<i>Tribulus terrestris</i>	1 part
Vridhdharu	<i>Argyrea speciosa</i>	1 part
Rasna	<i>Pluchea lanceolata</i>	1 part
Shatahva	<i>Anethum sowa</i> Kurz	1 part
Shati	<i>Hedychium spicatum</i>	1 part
Yamani	<i>Trachyspermum ammi</i>	1 part
Shunthi	<i>Zingiber officinale</i>	1 part
Guggulu	<i>Commiphora mukul</i>	12 parts
Ghee	Clarified butter	1 part

Fine powder of all these medicinal ingredients is added to Guggulu, compounded and made into tablets.

MATERIALS AND METHODS

Apparatus

For the evaluation of *in vitro* anti inflammatory activity Antech GT sonicator was used for the solubilization of the allopathic and the herbal formulation. An Incubator of Labline technologies was used for the incubation of the protein samples which is done before heating in electric heated water bath. A U.V spectrometer of Shimadzu, UV1700 was used for checking the extent of denaturation of protein in the heated samples.

Drugs and chemicals

All pure drugs Diclofenac, Aspirin, Paracetamol, Ibuprofen, Lornoxicam, Piroxicam were procured from Sigma Aldrich, India and other chemicals and reagents are purchased from SD Fine Chemicals, Mumbai of analytical grade.

Preparation of Guggulu Shodhana

Common impurities such as stone, bark, glass, etc. are withdrawn and then it is broken into small pieces. After that, the cleaned and broken pieces tied in a small cloth and are boiled in Dola-Yantra which contains Triphala decoction. The boiling of this mixture is done until Guggulu has turned into a soft mass. Later, it was retrieved onto a cloth and was spread onto a smooth wooden board which was previously smeared with ghee. It was dried in a dust free place and stored in an environment free from moisture and heat until it is further used for Trayodashang Guggulu preparation.

Method of Preparation of tablets

The above mentioned herbal drugs (table1) are dried and made into fine powders separately. These drugs and purified Guggulu put into the iron mortar and mixed well until a homogenous mixture formed. While mixing above mentioned; ghee also added for smoothening of the mixture. After the formation of mixture pills were made. Criterion to determine the final stage before making pills is that it should not stick to the fingers when rolled. The pills were dried in shade. These pills were kept in air tight containers. These pills were not exposed to sunlight.

EVALUATION OF ANTI INFLAMMATORY ACTIVITY BY *IN-VITRO* METHOD

Inhibition of protein denaturation method

The reaction mixture (5mL) contains 3mL of 1% egg albumin solution in phosphate buffered saline (PBS, pH 6.5) and 2mL of varying concentrations (200µg/ml and 1000µg/ml) of all the selected allopathic standard drugs. A Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2°C) in a BOD incubated for 30 min and then heated at 60° C for 10 min. After cooling, their absorbance was measured at 660 nm by using the vehicle as blank. The treatment is similar for determination of absorbance for herbal formulation.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition of protein denaturation} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$$\% \text{ Inhibition} = 100 \times (\text{Vt} / \text{Vc} - 1)$$

Where, Vt = absorbance of the test sample, Vc = absorbance of control.

The percentage inhibition of herbal drug is estimated and compared with standard drugs by solubilizing an entire tablet (in 10ml distilled water) and further it was sonicated for 30 minutes and filtered with Whatman filter paper. From the filtered solution a 2ml aliquot is added to the reaction mixture. Similarly, the same procedure is used for Bovine serum albumin to compare both the types of proteins using the same method (inhibition of protein denaturation method)

RESULTS AND DISCUSSION

In the present research, the anti inflammatory effect is evaluated against denaturation of egg albumin and bovine serum albumin.

The results are compiled in table 4 and table 5. However, prior to establishing the comparative study of the allopathic drugs with the herbal formulation, Diclofenac sodium was analyzed whether the drugs have concentration dependent inhibition of protein

denaturation or not (with both egg albumin and bovine serum albumin). The results of this primary analysis are shown in table 2, 3 and figure 1, 2.

Table 2: Relationship between concentration of Drug and % Inhibition of Protein Denaturation (Egg Albumin Method)

S. No	Concentration	Absorbance	% Inhibition
1	200 µg/ml	0.310	27.05
2	400 µg/ml	0.273	35.76
3	600 µg/ml	0.252	40.70
4	800 µg/ml	0.211	50.35
5	1000 µg/ml	0.170	60.01
6	Control	0.425	-

Table 3: Relationship between concentration of Drug and % Inhibition of Protein Denaturation (Bovine Serum Albumin Method)

S. No	Concentration	Absorbance	% Inhibition
1	200 µg/ml	0.030	46.42
2	400 µg/ml	0.026	53.57
3	600 µg/ml	0.023	58.92
4	800 µg/ml	0.019	66.07
5	1000 µg/ml	0.016	71.42
6	Control	0.056	

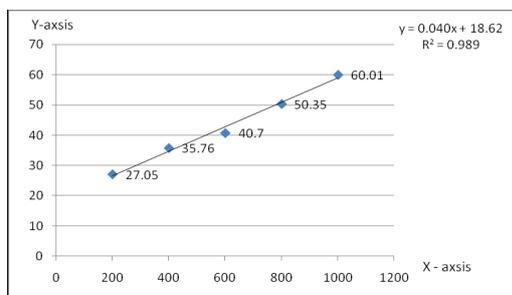


Figure 1: Linearity relationship between concentration of drug and %inhibition of protein (egg albumin) denaturation. X-axis: concentration (µg/ml), Y-axis: %inhibition of protein denaturation

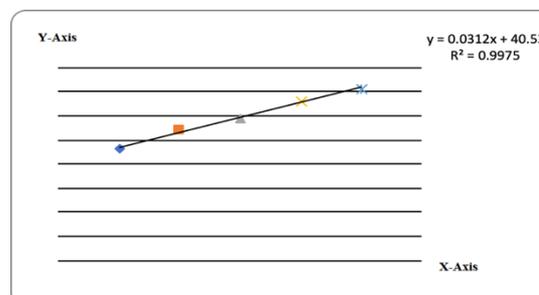


Figure 2: Linearity relationship between concentration of drug and %inhibition of protein (bovine serum albumin) denaturation. X-axis: concentration (µg/ml), Y-axis: % inhibition of protein denaturation.

Table 4: Results of % Inhibition with Egg Albumin Method

S. No	Drug name	Absorbance of Conc. (200µg/ml)	Absorbance of Conc. (1000µg/ml)	% Inhibition (200µg/ml)	% Inhibition (1000µg/ml)
1	Diclofenac sodium	0.308	0.17	27.18	59.81
2	Aspirin	0.334	0.296	26.6	42.9
3	Piroxicam	0.361	0.323	17.17	30.95
4	Ibuprofen	0.372	0.334	13.7	26.6
5	Lornoxicam	0.371	0.333	14	27
6	Paracetamol	0.406	0.368	4.18	14.94
7	Trayodashang Guggulu	1 full tablet = 0.283		49.46%	
8	Control	0.423			

Table 5: Results of % Inhibition with Bovine Serum Albumin Method

S. No	Drug name	Absorbance of conc. (200µg/ml)	Absorbance of conc.(1000µg/ml)	% inhibition with (200µg/ml)	% inhibition with (1000µg/ml)
1	Diclofenac sodium	0.028	0.017	50.00	69.64
2	Aspirin	0.039	0.019	30.3	66.07
3	Piroxicam	0.04	0.02	28.57	64.28
4	Ibuprofen	0.034	0.023	39.28	58.92
5	Lornoxicam	0.047	0.037	16.07	33.92
6	Paracetamol	0.046	0.040	17.8	28.57
7	Trayodashang Guggulu	1 full tablet = 0.018		67.85%	
8	Control	0.056			

Always there are complications while using animal subjects for *in vivo* pharmacological research, like ethical matters and lack of

rationale for the use of animals when there are some other methods that can also be used and investigated for the desired

results. Hence forth in the current study a bioassay by protein denaturation method was adopted as a part of *in vitro* estimation of anti-inflammatory property of the formulated Ayurvedic drug⁷. For inflammatory and arthritic conditions, denaturation of tissue proteins is one of the well proved causes. Hence it is known that auto antigen production in arthritic cases is due to protein denaturation^{8,9}.

That is why the chemical entities that have the ability to counteract against denaturation process can be potentially used as anti inflammatory drugs¹⁰. In table 2 and 3, the trend was observed that as we increased the concentration of the drug against control showed the absorbance slumped which represents the stabilization of proteins in the presence of anti inflammatory drug. So as a result the % inhibition of protein denaturation hiked up as we increased the concentration of the drug (Diclofenac sodium). From the table 4 and 5 it was clear that Diclofenac was more active than the other anti inflammatory counterparts having at least 50% inhibition even at the lowest concentration of 200µg/ml. These results were supported by conducting the same procedure on two different types of proteins, i.e., egg albumin and bovine serum albumin. The greater the turbidity of the sample, the greater is the denaturation of the protein that happened due to heating process¹¹.

Although, the turbidity of the test samples (herbal / allopathic drug), of all concentrations had been always less than that of control. This fall in turbidities is because of the decrease in concentration of herbal/allopathic drug in the reaction mixture, which resulted in decreased turbidity¹². Therefore, from the results of the present preliminary study, it can be concluded that herbal drug Trayodashang Guggulu possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein. Further definitive studies are necessary to deduce the mechanisms and constituents behind its anti-inflammatory actions. When 2ml of the herbal formulation extract was used in the reaction mixture, it showed a good % inhibition of protein denaturation than all the other allopathic drugs except Diclofenac sodium. It remained to be having the highest efficiency when compared to all the other drugs at both 200µg/ml and 1000µg/ml. This gives a conclusion that the Ayurvedic formulation has good, acceptable anti inflammatory properties.

While comparing the *in vitro* anti inflammatory activity of the individual herbal drugs in this formulation from the previously reported research literature review showed that the individual drugs have a considerable anti inflammatory activity but the combination is found to be better.

For instance, consider Ashwagandha from the results of the studies carried out previously. It was concluded that it possessed a marked anti inflammatory activity against denaturation of protein. The effect of Ashwagandha was more when compared to Diclofenac¹³. Similarly, when (*Tribulus terrestris* Linn) was tested for anti inflammatory activity 500µg/ml concentration of fruit kashaya showed a marked inhibition of almost 80% which is less than Diclofenac percentage inhibition¹⁴.

For instance, consider Ashwagandha from the results of the present preliminary study, it can be concluded that it possessed marked anti-inflammatory effect against the denaturation of protein *in vitro*. The present results exhibited a concentration dependent inhibition of protein (albumin) denaturation by the Ashwagandha extract. The effect of diclofenac sodium was found to be less when compared with the test extract. From this analysis, it can be concluded that the Ayurvedic formulation has effective anti inflammatory properties even though it is slightly less when compared to diclofenac. It can be evident that this Ayurvedic

formulation can be used for the inflammatory indications with the least number of side effects for a longer duration when compared to the allopathic formulations.

CONCLUSION

This is a preliminary study, we developed *in vitro* anti inflammatory activity of the Ayurvedic formulation Trayodashang Guggulu in comparison to commercially available allopathic drugs and further investigations are required to find an active component of the extract and to confirm the mechanism of action. The effect of concentration of test agent on the viscosity behavior of denatured protein dispersion requires further studies. The effect may be due to synergistic effect rather than single constituent. The present findings corroborated this property of Trayodashang Guggulu *in vitro*. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions both *in vivo* and *in vitro*.

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