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To Prepare a Poly Herbal Formulation Containing *Pluchea lanceolata* and *Vitex negundo* and Evaluate its Anti-Inflammatory Activity by Topical Application

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ABSTRACT

The aim of the study was to prepare a poly herbal formulation containing five herbs in the base of sesame oil and comparing its activity by using the carrageenan induced rat paw oedema model. The plants used in the study were *Pluchea lanceolata*, *Vitex negundo*, *Solanum xanthocarpum*, *Cleorodandrum phlomoides* and *Curcuma longa*. The oil obtained was applied topically to check the anti-inflammatory activity and comparing its efficacy with the standard which was marketed topical anti-inflammatory oil. The oil having more constituent of *Vitex negundo* in poly herbal formulation was found out to be having more potency in comparison to rest of the prepared samples as well as the marketed sample.

Keywords: Carrageenan; Anti-inflammatory; Topical application; Oedema; Potency.

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INTRODUCTION

Anti-inflammatory and analgesic are the most widely used class of drugs. Synthetically prepared drugs are their severe side effect of GIT distress and broncho constriction. The oil prepared from the herbal drugs will have no or less side effects as it will be applied topically and it will not undergo first pass metabolism.[5]

Vitex negundo (Family:-Verbaneceae) has been used in massage oil as well as in traditional ayurveda as a potent pain reliever. It is one of the most potent herbal drug for relieving pain. *Pluchea lanceolata*(Family:-Asteraceae) also popularly known as rasna has been used in ayurveda since early ages. The plants of *Pluchea* genus have been used traditionally used as astringent, antipyretic,anti-inflammatory, hepatoprotective, diaphoretic in fevers, smooth muscle relaxant, nervetonics, laxatives and for the treatment of dysentery.[1]

The plants of *Pluchea* genus have been used traditionally used as astringent, antipyretic ,anti-inflammatory, hepatoprotective, diaphoretic in fevers, smooth muscle relaxant, nervetonics, laxatives and for the treatment of dysentery. The conversion of different extracts into oil for the purpose of anti-inflammation has not been evaluated and so this experiment stands vast apart from the rest.[1,4]

The other plants like Curcuma Longa and Solanum Xanthocarpum give a synergistic effect to the activity of the Vitex negundo and Pluchea lanceolata. The transforming of active ingredients into the sesame oil was done by the traditional method. The turmeric has been used since ages for many purposes, mainly for the antiseptic and anti-inflammatory purpose. The anti-inflammatory action of the *Vitex negundo* leaves can be attributed to its flavonoids contents, which are known to act through inhibition of prostaglandin biosynthesis.[10,11,12]

MATERIALS AND METHODS:

Experimental Animals:

Albino Wister rats weighing 160-240gm were procured from the college animal house with the prior permission of CPCSEA. Each group consisted of six rats each. CPCSEA Number: IICP/UG/08-2011/03

Materials:

The formulation contains the known potent drugs in ayurveda. These herbs have been established as potent anti-inflammatory as well as analgesic action in the literature. The drugs to be used in preparation of the formulation [10,11]are:-

1. Vitex Negundo

2. Pluchea Lanceolata
3. Curcuma longa
4. Clerodandrum phlomoides
5. Solanum xanthocarpum

The formulation will contain a suitable base which will be either sesame oil or mustard oil.

Methods:

1. Preparation of formulation:-

The plant parts were obtained from the botanical garden from the campus. The plants were collected and washed thoroughly and cut into small parts. The respective plant parts were allowed to shade dried and then boiled in about 5 times of volume of water/solvent until 2 times of water was left. The heating was continued for over two hours until 2 times of water was left. The left over water/solvent extract was filtered and boiled with the suitable base (Sesame Oil) until all the water/solvent is evaporated. The oil obtained was filtered for any residual particles if seen and was stored in suitable container as amber color container. Three different oil were prepared, one having more amount of Vitex negundo (25% more than the actual concentration in other combination) and Pluchea lanceolata (25% more than the actual concentration in other combination) respectively.

2. Pharmacological activity :-

The anti-inflammatory activity of the prepared poly herbal formulation was checked with the help of carrageenan induced rat paw edema model [2,3,6,7,8,9]. The carrageenan was digested in saline for 24 hrs before injecting it into the rat. The oil was applied on the hind rat paw before thirty minutes of the injecting the carrageenan solution. The observations for Inflammation were taken at regular intervals of one hour, two hour and twenty four hour. Comparisons of data were made to evaluate the efficacy of the oil with the marketed preparation of anti-inflammatory oil.

Carrageenan induced rat paw edema model:-

Paw swelling, or footpad edema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. The protocol described in this unit uses carrageenan as the irritant to induce paw edema. Typically, test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. This model has long been used to assess the anti-inflammatory properties of agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin production.

Table 1: The Study Plan for the Carrageenan induced rat paw oedema model for different combinations.

Study plan					
Group	**I**	**II**	**III**	**IV**	**V**
Treatment	**Normal Control (carrageenan)**	**Standard treatment (voveran gel)**	**Treated with Combination I + Carrageenan[#]**	**Treated with Combination II + Carrageenan[$]**	**Treated with Combination III + Carrageenan***
Dose (mg/kg/day)	1 ml	3 ml	3 ml	3 ml	3 ml
Number of animals	4	4	4	4	4
Duration of treatment	Once	Once	Once	Once	Once
Route of administration	Subcutaneous	Topical Application +S.C.	Topical Application + S.C.	Topical Application + S.C.	Topical Application + S.C.
parameters to be evaluated	Paw Volume will be evaluated at different time interval (1, 2, 3 hr) up to 24 hour.				
Statistical Analysis	The data will be statistically evaluated by one wav analysis of variance followed by the comparison test to determine the level of significant difference between different combinations and optimizing the efficacy of the oil.				

[#]Combination I ---- Sesame Oil + extract of Pluchea *lanceolata + Vitex negundo.*
[$]Combination II --- Combination I + 25 % More Quantity of Vitex *negundo*
[*]Combination III -- Combination I + 25 % More Quantity Pluchea *lanceolata.*

Table 2: The measurement of the paw diameter by digital vernier calipers.

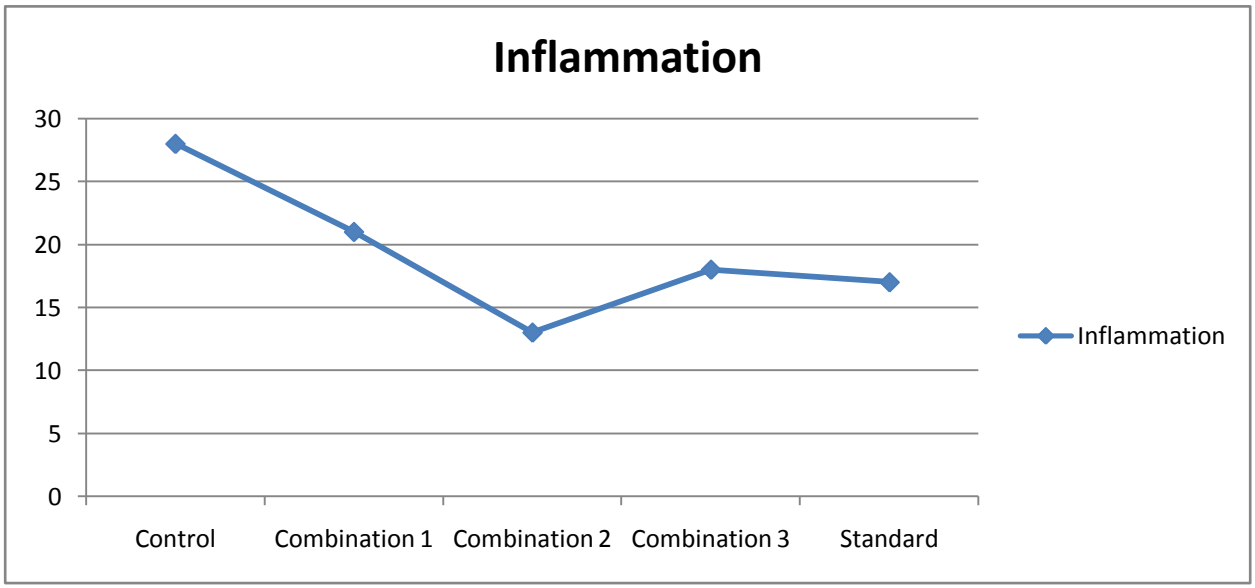
	Initial Readings			**Readings after one hour**			**Reading after 3 hours**			**Mean**
	Rat 1	**Rat 2**	**Rat 3**	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 1**	**Rat 2**	**Rat 3**	
Combination I	0.182	0.18	0.183	0.219	0.215	0.221	0.037	0.035	0.038	0.036667
Combination II	0.192	0.179	0.162	0.222	0.212	0.2	0.03	0.033	0.038	0.033667
Combination III	0.228	0.165	0.225	0.279	0.207	0.267	0.051	0.042	0.042	0.045
Control	0.199	0.174	0.166	0.257	0.231	0.224	0.058	0.057	0.058	0.057667
Standard	0.165	0.155	0.157	0.207	0.195	0.192	0.042	0.04	0.035	0.039

Explanation: The readings of inflammation were taken before injecting carrageenan(Intial readings) and then the readings were taken after 1 and 3 hours after injecting the carrageenan into the rat paw. The rat1, rat2 and rat3 denote the rats in the specific group used as test subject in each group.

RESULT AND DISCUSSION

The poly herbal oil obtained was tested for its anti-inflammatory activity on rats by Carrageenan induced rat paw edema. The Inflammation in rat paw was measured with the help of digital vernier calipers. The standard procedure of testing was followed. The prepared formulation was compared with the marketed sample of mahanarayan oil, especially the oil having more amount

of *Vitex negundo*. The poly herbal formulation was found to be having better activity than the marketed sample of Mahanarayan oil. The poly herbal formulation was found to be having better activity than the marketed sample of Mahanarayan oil. The oil having more percentage of *Vitex negundo* was found to be more active in alleviating the inflammation at lab level by the conducted experiment.



Graph 1: The above graph shows the percent inflammation exhibited after three hours of topical application.

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