

Investigations of the Prepared Polyherbal Ointment for its Anti-inflammatory Activity

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ABSTRACT

Inflammation is the defence response of the body to any injury or any other external agent that causes damage to the human body. Over many years there had been a rising concern for the inflammation that is caused due to the physiological stress and strain in the body due to the oxidative free radicals and oxidative damage. Medicinal plants have been used as the best treatment alternatives for the diseases in various medical systems in India and around the world. The scientific proof and validation are not authentic to that desired level. In this regard, herbs have been investigated for their pharmacological properties based on the medicinal claims and folklore claims. They had been worked by numerous amounts of research where the claims had been proven correct and published with scientific evidence. The ointment was prepared using the extracts of the plant Salvia and the activity was attributed to the phytochemicals, and by inhibiting the prostaglandin synthesis. The activity was comparable to the standard drug and the extracts and showed better activity. The activity exhibited by the ointment was very rapid and enhanced the healing of the paw edema, which was induced by carrageenan.



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INTRODUCTION

Inflammation is the defence response of the body to any injury or any other external agent that causes damage to the human body. Over many years there had been a rising concern for the inflammation that is caused due to the physiological stress and strain in the body due to the oxidative free radicals and oxidative damage [1]. Over 50% of the population suffers from inflammation of any type, either immunity generated or stress generated [2]. It is triggered

by many factors like any infection based, allergenic response, physical damage to the body and any other long term and chronic illnesses [3]. It is a complex process where the events of the inflammation are liberated which included pain mediators etc. that give rise to many other diseases.

Along with the inflammation, this results in the pain, the swelling also in the surrounding tissues. This leads to the generation of interleukins, cytokinins, TNFs etc. which furthermore induced the inflammation process [4]. Many drugs are available in the market to treat inflammations effectively, few of them are NSAID and SAID's which act by various mechanism and have multiple other pharmacological activities in the body. But they have serious side effects when used for prolonged periods like GI irritation, pain, perforations and peptic ulcers [5]. Medicinal plants have been used as the best treatment alternatives for the diseases in various medical systems in India and around the world. The scientific proof and validation are not authentic to that desired level. In this regard, herbs have been inves-

tigated for their pharmacological properties based on the medicinal claims and folklore claims. They had been worked by numerous amounts of research where the claims had been proven correct and published with scientific evidence.

In this present research, the plant *Salvia officinalis* which had been already proven to have the anti-inflammatory property of the topical application of the extract [6]. This is now used to prepare an ointment incorporating the extract into the ointment base and then investigated for the anti-inflammatory activity in two methods.

EXTRACTION

The aerial parts of the plant were collected and dried under direct sunlight. This was dried for three days and then powdered using a rotary mill. This was sieved, and the fine powder was collected. Then this powder was macerated using Ethanol 50% v/v with distilled water. The solution was macerated for five days with stirring and then filtered using a whatmann filter paper. The filtrate was collected, and the solvent was evaporated using a water bath, and the residue was turned into a thick paste which gave a yield of 19.8%w/w of the extract. This extract was stored in a desiccator for further use.

PREPARATION OF OINTMENT

General ointment base was selected for the preparation of the ointment. It was made of liquid paraffin and wax base. The base was yellow-coloured. The extracts were weighed according to Table 1 and incorporated into the ointment base by physical mixture method, and the quantity was made to the mark with ointment base.

Table 1: Preparation of PolyHerbal Ointment formulation

Sl no.	Material	Quantities
1	<i>Salvia officinalis</i> Extract	1g
2	Capsaicin	100mg
3	Menthol	Qs
4	Ointment base	10g

ANIMAL STUDY

Swiss mice were used to investigate for the study which weighed between 90-110 gm. They were kept in the propylene cages at $24 \pm 2^\circ\text{C}$ in a room with 12 hr day and night cycles with normal pellet diet and water. The rats were divided into four groups of 4 animals in each group. Group 1 was treated as a normal group with the induction of the inflammation and were given plain distilled water via the

oral route. This group was considered as a negative control group. Group II animals were administered with the standard drug, Indomethacin drug at a dose of 15mg/kg body weight. Group III and Group IV were considered as test groups which received an extract of 200mg/kg and the ointment equivalent to the 200mg/kg of the extract. The standard drug and the extract were given in oral route, and the ointment was given via the topical route application which covered the inflamed surface in any method.

The potency of the ointment was tested using carrageenan-induced paw edema method wherein the mice were induced with edema using carrageenan as an inducing agent [7, 8]. The animals were administered with the extracts and standard drugs, and after 30 min of the treatment, induction of inflammation was made. 0.1 ml of 1%w/w of carrageenan was suspended in the normal saline solution and injected into the right plantar region of the hind paw of the animals. This was left alone for a few mins, and the group IV was mice were applied with the ointment at the site of inflammation. The volume of the paws was checked in the regular timings of 15mins, 30 mins, 1hr, 1.5hrs and 2 hrs. The percentage inhibition of the edema of the paw and the paw volumes were measured, and the formula was applied for calculating the percentage inhibition.

Percentage of inhibition of edema-%I = $(1 - V_{\text{test}}/V_{\text{control}}) \times 100$

COMPARISON OF ACTIVITY

The anti-inflammatory activity of the prepared polyherbal ointment was investigated using the paw edema and volume. There was a significant induction of the paw volumes of the mice in the control group. The induction of inflammation was successful. The lowering of the paw volume was achieved with standard drug indomethacin and the extracts. But the significant lowering was achieved with the ointment. The inflammation is the response of the body to the external stimulus and damage-causing stimulus. When this stimulus was chronic and continuous, then it becomes a disease and leads to further damage [9, 10].

The mechanism of the action of the extracts was due to inhibition of the histamines and prostaglandins, which induced the inflammation in the experimental mice [11, 12]. The extract and ointment showed better activity in carrageenan method, which was compared to the standard indomethacin so it can be presumed that the mechanism was similar to that of the standard. Generally, all the NSAID drugs have an inhibiting property of the PG and cytokinin [13]. Thus the mechanism attributed to the extracts was also the same.(Table 2)

Table 2: Anti-inflammatory Property of the Polyherbal Ointment

Drugs	0min	15min	30min	60min	120min	180min	%inhibition
	Volume (ml)						
Distilled water	0.74±0.12	1.26±0.14	1.53±0.07	1.92±0.16	1.85±0.13	1.67±0.10	-
Indomethacin Extract	0.86±0.06	1.13±0.11	1.17±0.18	1.14±0.19	0.72±0.24**	0.49±0.05**	70.73
Ointment	0.78±0.10	1.42±0.09	1.29±0.15	1.56±0.17	0.91±0.12*	0.80±0.26*	57.32
	0.91±0.13	1.16±0.20	1.18±0.32	1.21±0.4	0.77±0.45**	0.58±0.08**	66.46

The ointment showed a better activity compared to the extract and standard drug. This may be due to the penetration enhancer drug capsaicin, which is present in the ointment. There is menthol also in the ointment, which also made healing quicker and reduction in the paw volume of the mice.

CONCLUSION

The ointment was prepared using the extracts of the plant *Salvia* and the activity was attributed to the phytosterols, and by inhibiting the prostaglandin synthesis. The activity was comparable to the standard drug and the extracts and showed better activity. The activity exhibited by the ointment was very rapid and enhanced the healing of the paw edema, which was induced by carrageenan.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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