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ESTIMATION OF ANTIARTHRITIC ACTIVITY AND INHIBITION OF PRO-INFLAMMATORY CYTOKINES BY A POLYHERBAL TABLET FORMULATION IN HISTAMINE-INDUCED ARTHRITIS IN RATS

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Abstract

Objective: The objective was to evaluate the anti-inflammatory and antiarthritic activity of Polyherbal tablet formulation in Histamine-induced arthritis in rats.

Materials and Methods: The antiarthritic activity and pro inflammatory cytokines inhibition by Polyherbal tablet formulation was evaluated using Histamine-induced arthritis models, at doses of 100, 200 mg/kg body weight. The anti-inflammatory activity of Polyherbal tablet formulation of was assessed using a digital plethysmometer in the Histamine induced paw edema model. Paw volume were measured at regular intervals using a micrometer screw gauge. Serum samples were collected for the estimation of pro-inflammatory cytokines. Diclofenac (20 mg/kg body weight) was used as the standard drug in both models. Acute and chronic toxicity studies were conducted to assess the safety of the test drug.

Results: Treatment with Polyherbal tablet formulations of *Trapa bispinosa, Cassia uniflora, Bosvellia serrata and Cissus quandragularis* 100, 200 mg/kg resulted in a dose-dependent reduction in paw edema and paw thickness in the Histamine-induced paw edema arthritis models, respectively, compared to the standard group. Polyherbal tablet formulation of *Trapa bispinosa, Cassia uniflora, Bosvellia serrata and Cissus quandragularis* 100, 200 mg/kg also showed a dose-dependent decrease in the expression of pro-inflammatory mediators compared to the standard group.

INTRODUCTION

Rheumatoid arthritis is a systemic inflammatory disease that manifests itself in multiple joints of the body. The inflammatory process primarily affects the lining of the joints (synovial membrane), but can also affect other organs. The inflamed synovia leads to erosions of the cartilage and bone and sometimes joint deformity. Pain, swelling, and redness are common joint manifestations. The prevalence of RA in the Indian subcontinent is 0.4-0.6% of the population. The epidemiological ratio of arthritis in females to males is 3:1, and the prevalence is 1% of the world population[1].

The pathogenic mechanisms of synovial inflammation are likely to result from a complex interplay of genetic, environmental, and immunologic factors that produce dysregulation of the immune system and a breakdown in self-tolerance. It is caused by a number of pro-inflammatory molecules released by macrophages, including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes, and cytokines [2,3]. The regulation of these mediators

secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like COX and LOX are potential targets for chronic inflammatory conditions.

Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal agents, and immune suppressants are usually used as RA treatments. However, their side effects and toxicity call for alternative, safer, and more effective natural product-based drugs. There is now a growing concern for the development of new safe, potent, and less toxic antiarthritic drugs. Hence, there is a need to explore more naturally available alternatives so that their therapeutic values can be assessed and expanded [4].

The objective of the present study is to formulate a polyherbal formulation (PHF) and evaluate its antiarthritic potential in animals. The PHF was formulated using herbs that have known antiarthritic effects at a particular ratio to enhance the pharmacological activity of individual herbs and reduce the dose of single plant extract. In Ayurveda, two principles are used for drug formulation, namely, single herb or more. Extracts of *Trapa bispinosa*, *Cassia uniflora, Bosvellia serrata and Cissus quandragularis* were used in the PHF. In the traditional system of Indian medicine, combined extract of individual plants is used rather than individual ones to achieve maximum therapeutic efficacy [5] Histamine induced arthritis model was used to evaluate the anti arthritic activity.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats (150–200 g) from SGRS College of Pharmacy , Pune , were used in the study. Animals were housed under standard laboratory conditions at25°C ± 2°C in groups of three with free access to food and water ad libitum . They were acclimatized to the laboratory conditions for a period of 5 days before the study. The study was carried out in the Department of Pharmacology after approval of the Institutional Animal Ethics Committee, Protocol approval number was SGRS/IAEC/05/2021-22

Drugs and chemicals

Polyherbal tablet was prepared by using the extracts of *Trapa bispinosa, Cassia uniflora, Bosvellia serrata and Cissus quandragularis.* The crude drugs were purchased from Gokuldas and Company , PuneDiclofenac sodium 20 mg / kg was used as standard drug . Histamine and Twin 80 was purchased from Research lab, Pune .

Histamine induced paw edema in Rats

Wistar rats weighing between 200-250 gm were selected and divided into four groups. Food was withheld with water *ad libitum* 12 hrs before the administration of drugs. Animals were treated orally as follows: control group received vehicle, 2 test groups received two different doses of test drug 100, 200 mg/kg p.o. and standard group received marketed drug (diclofenac 20 mg/kg) for comparison. All animals received injection of 0.1 ml of 1% histamine in sub-plantar region ½ or 1 hr after drug treatment. Inflammation was measured using plethysmometer, at an interval of 0, 1, 2, 5 hours after Histamine injection [6]

Detection of circulating cytokines

The blood samples were collected and send to Maharashtra Animal and Fishery University, Department of Veterinary , Krantising Nana Patil College of Veterinary Science, Pune , for analysis Circulating tumor necrosis factor-alpha (TNF-α), IL-6 and HMGC 1 level was estimated by ELISA.

Toxicity studies

Evaluation of oral acute toxicity of Polyherbal tablet formulation of *Trapa bispinosa, Cassia uniflora, Bosvellia serrata and Cissus quandragularis* was carried out according to the Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals-425.[7,8]

Statistical analysis Difference between groups was compared using one-way ANOVA followed by Dunnett's Multiple Comparison.P <0.05 was considered significant.

RESULTS

Composition of polyherbal tablet formulations

HPMC, xanthan gum, lactose, microcrystalline cellulose and magnesium Stearate were the excipients selected for the tablet formulations.

All the ingredients were weighed in the required quantity and mixed in the ascending order by weight. Water was added slowly to the mixture just enough to form a dough mass. This dough mass was passed through 18# mesh sieve to obtain granules. The granules were dried and passed through 80# mesh sieve.

After drying, the granules were lubricated with magnesium state using octagonal blender with 25rpm speed for 10 mins.

These granules were compressed into tablets using flat punches [6-8]. The composition of the polyherbal tablet formulation is mentioned in Table 1.

Four different compositions each for 100mg and 200 mg formulation were prepared. By keeping the drug amount constant, the excipient were varied in order to select the best composition among them. Total 8 batches were prepared among these F4 and F8 formulation was selected for the study.

Table 1: Composition of Polyherbal tablet formulation

Name of the ingredient	**Quantity in mg**							
	Formulation code for 100 mg tablet				**Formulation code for 200 mg tablet**			
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Trapa bispinosa	25	25	25	25	50	50	50	50
Cassia uniflora	25	25	25	25	50	50	50	50
Cissus quandragularis	25	25	25	25	50	50	50	50
Boswellia Serrata	25	25	25	25	50	50	50	50
HPMC	10	20	30	50	20	40	60	100
Xanthan gum	90	80	70	50	180	160	140	100
Lactose	5	10	15	20	10	20	30	40
MCC	40	35	30	25	80	70	60	50
Magnesium Stearate	5	5	5	5	10	10	10	10

Acute Oral Toxicity Study

Determination of LD_{50}

Toxicity study was carried out using a starting dose of 2000 mg/kg body weight. Individual animal observations were made after dosing at least once in the first 30 min., on occasion in the first 24 h, with particular focus on the first 4 h.. OECD Guidelines, No. 425(para-36). A single

dose of 5000 mg/kg produces some lethargic effects on the mice. So the LD50 is lesser than the 5000 mg/kg that is 2000 mg/kg. All the five animals were survived. Thus, the one tenth dose of 2000 mg/kg i. e. 200 mg/kg was selected as a therapeutic dose and the sub-therapeutic and super-therapeutic dose were selected as 100 mg/kg and 200 mg/kg respectively . The observations of acute toxicity studies are listed in Table 2.

Table 2 : Behavioral and physical observations for acute toxicity studies

Observation	**30 mins**	**4 hrs**	**24 hrs**	**14th day**
Body weight	No change	No change	No change	No change
Preterminal deaths	Absent	Absent	Absent	Absent
Cage side observation	Normal	Normal	Normal	Normal
Motor activity	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent
Piloerection	Absent	Absent	Absent	Absent
Righting reflex	Present	Present	Present	Present
Lacrimation	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Respiration	Normal	Normal	Normal	Normal
Skin Colour	Normal	Normal	Normal	Normal
Diarrhoea	Absent	Absent	Absent	Absent
Corneal reflex	Normal	Normal	Normal	Normal
Pinnal reflex	Normal	Normal	Normal	Normal
Grooming	Absent	Absent	Absent	Absent
Sedation	Normal	Normal	Normal	Normal
Excitation	Normal	Normal	Normal	Normal
Aggression	Normal	Normal	Normal	Normal

There were no morbidity and mortality observed for polyherbal formulation treated animals upto 2000 mg/kg. From the acute toxicity study, the LD50 cut-off dose for extracts was found to be 1000 mg/kg body weight. Hence, the therapeutic doses were taken as 100 mg/kg and 200 mg/kg body weight.

Effect of polyherbal formulation tablet on Paw Volume Changes (in ml) in Histamine Induced Paw Edema in Rats(1 Day Treatment) is expressed through Table 3 and figure 1

Table 3 : Effect of polyherbal formulation tablet on Paw Volume Changes (in ml) in Histamine Induced Paw Edema in Rats(1 Day Treatment)

Groups	**Treatment**	**Mean Paw edema (ml) ±SEM**			
		Before injection	**1hr**	**3hr**	**5hr**
Group I Normal Control	Tween 80 (1%)	1.16±0.185	1.85±0.240	1.76±0.21	1.51±0.214
Group II Std Drug	Diclofenac sodium 20	0.55±0.114	0.73±0.105*	0.83±0.1406*	0.73±0.125*
Test Group III TCBC 100 mg/kg	TCBC 100 mg/kg	0.86±0.149	1.78±0.157*	1.73±0.211	1.49±0.207
Test Group IV TCBC 200 mg/kg	TCBC 200 mg/kg	0.71±0.179	1.55±0.095*	1.46±0.115*	1.32±0.070*

Whereas, SEM=Standard Error Mean All values are expressed as mean ±SEM (n=6) using the ANOVA followed by Dunnet's test. Result considered as significant at * *p <0.05, ** p <0.01 compared with control Group.*

TCBC 100 mg/kg showed decrease in paw edema at all intervals but significantly (p <0.01) only at 1 hr compare to normal control, however, 200 mg/kg body wtp.o significantly(p <0.01) decreased paw edema at 1st , 3 rd and at 5th hr (0.05) intervals compare to normal control

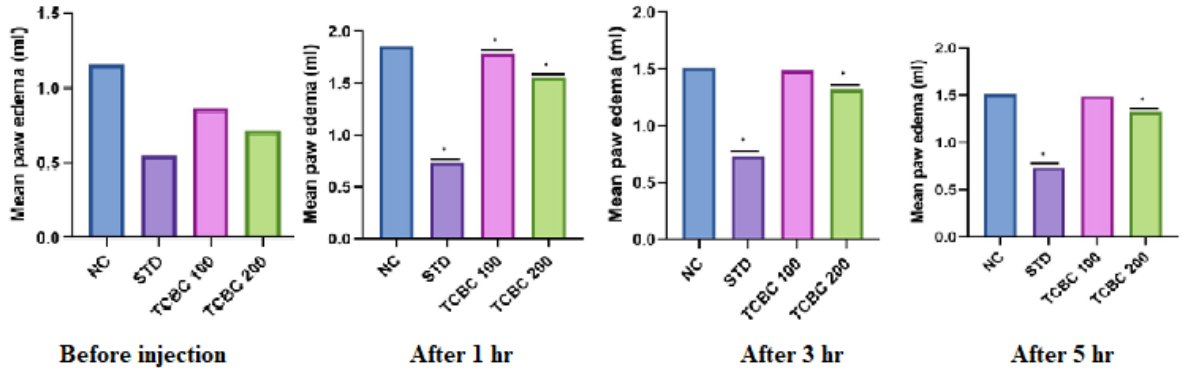

Figure 1: Mean paw edema

Effect of PHF on IL-1β, TNF-α, and HMGB-1 in Histamine -induced acute inflammation model

The findings demonstrated that the control group's levels of the pro-inflammatory cytokines IL-1, TNF-, and HMGB-1 were considerably (#p< 0.05) higher than those of the normal group (Table 7.21). Because of this, oral administration of PHF 100 mg/kg to the pretreatment group caused a significant (**p<0.01) decrease of TNF- over the first 1 to 5 hours as compared to the control group. After the administration of Histamine for 1 to 3 hours, the level of IL-1 was considerably lower in the PHF group compared to the control group. When PHF was used as a pretreatment, HMGB-1 expression was noticeably lower than it was in the control group[`9]

Table 4: Levels of serum pro-inflammatory cytokine in Histamine -induced inflammation in rats

Cytokine	**Group**	**Histamine induced inflammation**		
		1h	**3h**	**5h**
TNF-α (pg/ml)	Normal	23.65± 2.67	23.65± 2.67	23.65± 2.67
	Control	55.46±13.33$^{\#}$	62.50±6.93$^{\#}$	46.25±9.6$^{\#}$
	TCBC 200	31.80±4.37**	40.10±6.23**	35.75±7.91*
IL-1β (pg/ml)	Normal	9.5±4.11	9.5±4.11	9.5±4.11
	Control	51.4±10.6$^{\#}$	46.5±5.2$^{\#}$	29.7±5.8$^{\#}$
	TCBC 200	22.9± 2.5**	27.5±7.3**	36.8±2.2
HMGB-1 (µg/ml)	Normal	1.16±0.08	1.16±0.08	1.16±0.08
	Control	2.48±0.78$^{\#}$	2.58±0.53$^{\#}$	3.91±0.55$^{\#}$
	TCBC 200	2.092±0.18	3.00±0.22	2.51±0.38*

Data were expressed as mean ± SD of 6 rats in the experimental group. $^{\#}p<0.05$ vs Normal group, *p<0.05; **p<0.01 vs Control group by Two-way RM ANOVA followed by Tukey's post hoc test.

Effect on Serum pro-inflammatory cytokine concentrations in Histamine-induced paw inflammation

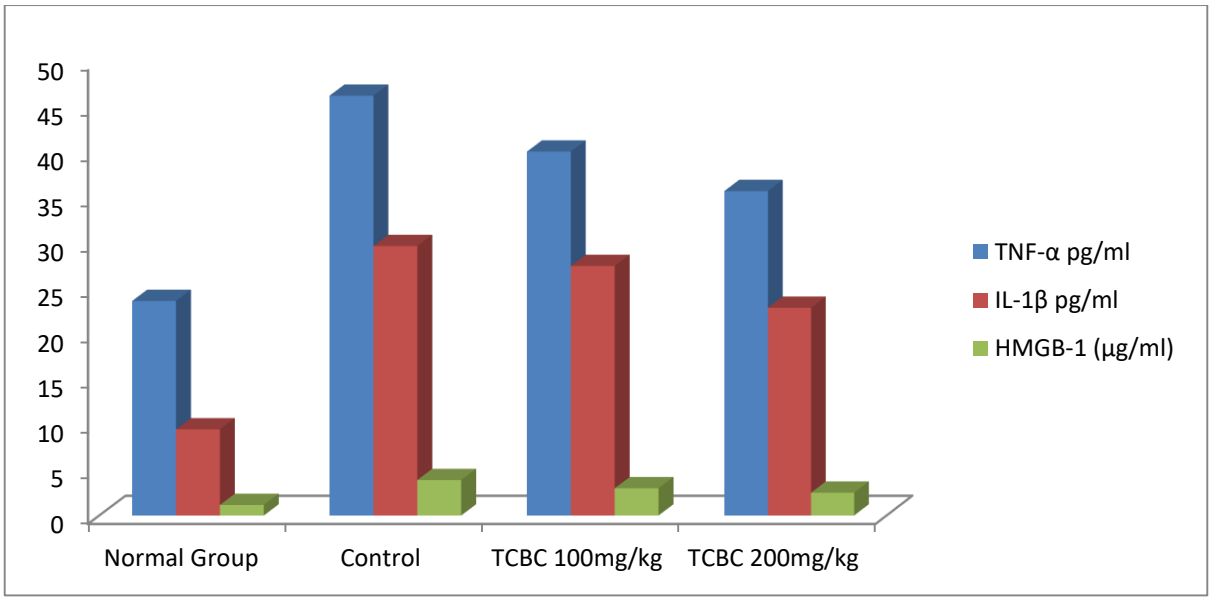

Figure 2: Effect on Serum pro-inflammatory cytokine concentrations in Histamine-induced paw inflammation

DISCUSSION

Polyherbal tablet formulation used for the study comprises four herbal medicinal plants which were *Trapa bispinosa* , *Cassia uniflora* , *Bosvellia serrata* and *Cissus quandragularis.*

It was found to have an oralLD50 above 2000 mg/kg. Chronic administration of Polyherbal tablet formulation also did not produce any pathological changes in tested animals, thus demonstrating its safety on long-term administration

Polyherbal tablet formulation has shown to be effective in reducing paw volume in Histamine induced arthritis in Rats. It is also found that the Polyherbal tablet formulation used effectively reduces the pro inflammatory cytokines such as TNF-α (pg/ml), IL-1β (pg/ml), HMGB-1 (µg/ml) levels in the rats.

The probable mechanism might be immune modulatory activity of individual constituents present in Polyherbal tablet formulation mainly by *Trapa bispinosa* , *Cassia uniflora* , *Bosvellia serrata* and *Cissus quandragularis* have already shown disease modifying activity by inhibiting the pro-inflammatory cytokines.

Based on these finding it is concluded that the Polyherbal tablet formulation containing *Trapa bispinosa* , *Cassia uniflora* , *Bosvellia serrata* and *Cissus quandragularis* has potential anti arthritic activity and potent inhibitor of pro inflammatory cytokines. As the natural source of medicines could be further explored for the safer alternative in the treatment of Arthritis.

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