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Der Pharmacia Lettre, 2016, 8 (3):182-188
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Pharmacological screening of poly-herbal combination for anti acne activity

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ABSTRACT

The aim of the present study was an attempt made in our laboratory to research for herbal-based anti-inflammatory products known to have beneficial effects of anti-inflammatory disorders in traditional medicines. The plant materials used for the anti-inflammatory activity were Curcuma longa, Bombax ceiba, Ficus bengalensis and Lens culinaris. Various in-vitro and in-vivo models have been proposed as being able to detect anti-inflammatory effect. A few of these methods have been symmetrically evaluated for their potential usefulness in screening beneficial effects on acne. The result obtains by MECL, MEBC was 50.06% suppression where as other combination MECL and MEFB are 45.97% and MEFB and MEBC was 35.50% and a combination of MECL, MEFB and MEBC was 31.35 % suppression in paw oedema volume which is extremely significant as comparable to standard drug indomethacin 10mg/kg.

Keywords: anti-inflammatory, P. acne, anti acne activity, *Curcuma longa*, *Bombax ceiba*, *Ficus bengalensis* and *Lens culinaris.*

INTRODUCTION

Acne is an affection that concern 80% of young people in the world with the significant impact on their quality of life. A peak of frequency was noted in the age group of 21-25 years in the 2 sexes. The prevalence of disease results in 20% of all visits to dermatologist belonging for acne [1]. Acne vulgaris is a most common dermatological disorder of pilosebaceous units and topical therapy is recommended for the management of acne with comedolytic, anti-inflammatory agents, along with antimicrobials [2]. *Propionibacterium acnes (P. acnes)* play an important role in the pathogenesis of acne inflammation by producing polymorphonuclear leukocyte and monocyte or macrophage to produce proinflammatory mediators. Moreover, *P. acnes* can also induce follicular keratinocytes to release interleukin-1, which causes keratinocytes to proliferate and contributes to the formation of the preclinical micromedo. Therefore, the compounds for targeting acne vulgaris should be able to inhibit *P. Acnes* [3].

The complexity of the inflammatory process and the diversity of the drugs that have been found effective in modifying this process have resulted in the development of numerous methods of assay for distinguishing anti-inflammatory substances. Various *in-vitro* and *in-vivo* models have been proposed as being able to detect anti-inflammatory effect. A few of these methods have been symmetrically evaluated for their potential usefulness in screening beneficial effects on acne. An attempt was made in our laboratory to search for herbal-based anti-inflammatory products known to have beneficial effects for inflammatory disorders in traditional medicines. Predominantly, however, these studies are aimed to find new drugs against acne and other inflammatory disorder.

Since the etiology of acne is considered to be largely immunological, special tests have been developed to investigate various immunological and allergic factors.

MATERIALS AND METHODS

Preparation of extracts

The plant materials of *Curcuma longa L, Lens culinaris monech , Bombax ceiba L,* and *Ficus benghalensis L.* were collected from Bhopal M.P on December 2013. The materials were authenticated by Dr. Zia UL Hasan, Department of Botany, Saifia Science College, Bhopal (M.P.) and preserved in the herbarium of the College. Specimen Voucher no: **449/Bot./Saifia/13.** was assigned for further reference. The air-dried and powdered defatted marc of the drug was subjected to extraction with methanol. The crude was soaked in 2.5 L of methanol for 6-7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a wool plug followed by Whatman filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator.

Animal and Chemicals

Animals

Albino rats 100-150 gm of either sex were used for this experiment. The animals were housed in the polypropylene cages in animal house, **TIT Pharmacy, Bhopal M.P.** and provided with food and water *ad libitum*. The research was conducted in accordance with the ethical rules on animal experimentation approved letter: - by ethical committee Reg. No. (TIT/ IAEC/831/P'col/2014/45).

Chemicals

Croton oil was purchased from Sigma-Aldrich Inc., INDIA. Solutions in acetone are made fresh as required. Solutions in acetone are made fresh as required. It is light-sensitive, Toxic by inhalation, in contact with the skin. It is irritating to the eyes and respiratory system. It should be stored at room temperature.

Acute toxicity studies (LD_{50})

The acute oral toxicity studies of MECL, MEBC, MEFB and MELC was determined using Swiss albino mice. The animals were fasted for 3 hours prior to the experiment and were administered with a single dose of extracts dissolved in 5% gum acacia (dose range from 500-2000 mg/kg at various dose levels) and observed for mortality up to 48 hours (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 423. All the animals were also observed for long-term toxicity (14 days). [4].

Experimental design:-

The rats were divided into control group (group – I), standard drug treatment group (group –II), Methanolic extract MECL, MEBC, MEFB, and MECL group (group III, IV, V, VI) and comparising 6 rats each. Group – I rats received only the vehicle (5% gum acacia, 1 ml/100 gm). Rats of group – II were treated orally with 10 mg/kg of Indomethacin as a standard drug. Rats of group- III, IV, V, VI, were treated orally with (250 mg/kg) MECL, MEBC, MEFB, and MECL respectively by orogastric cannula.

Preparation of test and standard drug solution

a) Croton oil: Croton oil solution was prepared by using acetone (40 µg in 10 µl acetone).
b) Standard drug: Standard drug indomethacin was used for topical administration.
c) Test sample: Methanol extract of selected plant was used for evaluation.

Group test animals

Six groups of 6 rats each were selected for different treatment groups.
Group 1: One is served as control.
Group 2: Animals of groups 2 received standard drug indomethacin topically and p.o.
Group 3, 4, 5 and 6: Similarly groups 3, 4, 5 and 6 received a methanol extract in form of solution.

Method for testing acute and sub acute inflammation [5].

Cotton pellets induced granuloma in rats:

The animals were divided into 6 groups of 6 animals in each group. The rats were anesthetized with Diethyl ether and sterile cotton pellets weighing 20± 1mg were implanted subcutaneously into the groin region of each rat. Group I served and received the vehicle (5% gum acacia, 1 mi/100gm). MECL, MEBC, MEFB and MELC (250 mg/kg)

was administered orally to groups III to V animals for 7 consecutive days from the day of cotton pellet implantation. Group II animals received Indomethacin at a dose of 10 mg/kg for the same period. On 8th day, the animals were anesthetized and the pellets together with the granuloma tissue were carefully removed and made free from extraneous tissues. The wet pellets dried in an oven at 60ºC for 24 hrs. to the pellets weight, after that the dried pellets were weighed again. An Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The anti-proliferative effect of MECL, MEBC, MEFB, and MECL was compared with control.

$$\% \, Inhibition = \frac{Wc-Wt}{Wc} \text{ x } 100$$

Where, we represents the pellet weight of the control group animals and WT represents the pellet weight of the drug treated group animals [6].

Reagent composition

A. Sterilized cotton pellet (Each weighing 20 mg implanted subcutaneous{s.c.})
B. Indomethacin 10 mg/kg, p.o. prepared as a stock solution.

Paw oedema in Albino Rats: The rats were divided into 6 groups (n=6). The different groups were treated orally with MECL, MEBC, MEFB, and MECL (250 mg/kg), indomethacin 10 mg/kg and vehicle control (5% gum acacia, 1 ml/ 100gm). The MECL, MEBC, MEFB and MECL standard drug and vehicle control were administered 1 hour prior to an injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub planter of each rat. The paw volume was measured initially and then at 1, 2 and 3 hours interval after the carrageenan injection by using plethysmometer. The anti-inflammatory effect of MECL, MEBC, MEFB and MECL was calculated by the following equation.

$$\% \text{ Oedema Inhibition} = \frac{Vc-Vt}{Vc} \text{ x } 100$$

Where, Vt represents the paw volume in drug treated animals and Vc represents the paw volume of control groups animals. [7].

Anti-inflammatory evaluation of Croton oil-induced ear oedema method

Albino rats were selected to groups of six rats each. The effect of oedema on acute topical inflammation was evaluated by a modification of the methods . Oedema was expressed as the increase in ear weight and thickness due to the inflammatory challenge [8],[9]. Ear thicknesses were measured before and after induction of the inflammatory response by using a Vernal caliper. The Vernal caliper was applied near the tip of the ear and the thickness was noted in µm. To minimize variation due to technique, a single investigator performed the measurements throughout any one experiment. Oedema was induced in each rat by applying a solution of croton oil in acetone (40µg/10µl) on the inner surface of the right ear. 1 h after treatment with croton oil applies prepared methanolic extract was applied topically. 6 h later, the rats were sacrificed by anesthesia using ether, then cervical dislocation and both ears cut off and weighed and also measured their thickness. Oedema was determined by measuring the difference in weight and thickness of ears for each mouse**.** [10].

Table 1: Cotton pellet induced granuloma in rats

Treatment	Dose (mg/kg	Dry weight of cotton pellets (mg)	% Inhibition
Control (Vehicle)	-	91.5± 1.44	-
Standard Drug (indomethacin)	10mg	29.5± 0.28***	67.75
MECL	250mg	42±0.40***	54.09
MEBC	250mg	53.75±0.80***	41.25
MEFB	250mg	50.5±1.02***	44.80
MELC	250mg	72±0.40	21.31

*The values are expressed as mean± S.E.M, n=6 in eac h group, *** p≤0.001, ** p≤0.01, * p≤0.05 ns-non significant when compared with the control group.*

Table 2: % inhibition of carrageenan induced rat Paw oedema in rats

Treatment	Dose (mg/kg	Odema volume in after 3 hour	% Decrease in oedema volume
Control (Vehicle)	-	0.85±0.07	-
Standard Drug (indomethacin)	10mg	0.15±0.02***	82.35
MECL	250mg	0.32±0.06***	61.76
MEBC	250mg	0.44±0.14**	48.00
MEFB	250mg	0.35±0.04***	58.82
MELC	250mg	0.53±0.05*	37.64

*The values are expressed as mean± S.E.M , n=6 in ea ch group, *** p≤0.001, ** p≤0.01, * p≤0.05 ns-non significant when compared with the control group.*
Methanolic extracts of Curcuma longa. (MECL)
Methanolic extracts of. Bombax ceiba (MEBC)
Methanolic extracts of Ficus benghalensis (MEFB)
Methanolic extracts of Lens culinaris (MELC)

Parameters Studied
After 5 h of sample application the animals of all groups were scarified by cervical dislocation and their ears were separated from the study of different parameters.

Determination of ear weight difference
Difference in the weight of ear of the all group rats was shown in **Table 3.1**

Table 3: Weight variation of ear of experimental animals

S. No.	Treatment	Weight of ears (mean±SEM)
1.	Control	0.072±0.010 µg
2.	Positive control	0.035±0.008 µg
3	Methanol extract of *Curcuma longa*	0.044±0.006 µg
4.	Methanol extract of *Lens culinaris*	0.062±0.005 µg
5.	Methanol extract of *Bombax ceiba*	0.051±0.010 µg
6.	Methanol extract of *Ficus bengalensis*	0.049±0.012 µg

Number of animals per group = 6

Determination of Ear Thickness
Ear thickness was measured using vernal calipers. Calculate the mean thickness of the ears of all groups and compared with that of the croton oil controls given in Table 4

Table 4: Thickness variation of ear of experimental animals

S. No.	Treatment	Thickness of ears (mean±SD)
1.	Control	150±16.02 µm
2.	Positive control	69±08.08 µm
3	Methanol extract of *Curcuma longa*	82±09.11 µm
4.	Methanol extract of *Lens culinaris*	115±16.12 µm
5.	Methanol extract of *Bombax ceiba*	91±12.12 µm
6.	Methanol extract of *Ficus bengalensis*	89±11.01 µm

Number of animals per group = 6

Procedure for histopathological evaluation
A small piece of ear of experimental animal was cut into 5-10 µm thick section by stratum and put on the slide. The tissue was fixed with 70% ethanol/acetone for approx 5 min. Then the slides were washed with distilled water. Staining was done with hematoxylin for 3 min. Excess of hematoxylin was removed by rinsing the slides with distilled water and then with tap water. Distaining was done with the help of alcohol for 8 - 12 times. Then the slides were rinsed with distilled water and excess water was blotted from the slides, then the slides were dipped in eosin for 45 sec, then in 95% ethyl alcohol for 3 min and finally in the xylene for 5 min. Mounting of slides was done using dpx mount slides were observed under microscope.

COMBINATION OF SELECTED HERBS
Preparation for the combination of selected drugs:-
On the bases of results of anti-inflammatory of individual selected plant extract (methanol) carried out reveals that methanol extracts of *Curcuma longa* and *Ficus bengalensis* have shown good anti-inflammatory activity in

comparisons to other methanol extract. After the study of anti-inflammatory activity of methanol extract of selected plants, Methanol extract of *Curcuma longa, Ficus bengalensis* and *Bombax ceiba* was selected for preparation of combination.

It was believed that the combined effect of these drugs may give more significant effect as anti-inflammatory. Hence it was decided to carry out the combination study of these drug samples and also for their anti-inflammatory effect. The combinations of different plants methanol extract (*Curcuma longa, Ficus benghalensis, Bombax ceiba*) were prepared in 500 µl ethanol for evaluation of their anti-inflammatory activity.

Table 5: Combination of extracts for % inhibition of carrageenan induced rat Paw oedema in rats

Treatment	Dose (mg/kg	Odema volume in after 3 hours	% Decrease in oedema volume
Control (Vehicle)	-	0.82±0.06	-
Standard Drug (indomethacin)	10mg	0.12±0.04***	85.36
MECL+ MEBC	250mg+ 250mg	0.29±0.09***	64.63
MECL+MEFB	250mg+ 250mg	0.38±0.17**	53.65
MEFB+MEBC	250mg+ 250mg	0.55±0.04***	32.92
MECl+MEFB+MEBC	200mg+ 200mg+ 100mg	0.48±0.08*	41.46

*The values are expressed as mean± S.E.M , n=6 in each group, *** p≤0.001, ** p≤0.01, * p≤0.05 ns-non significant when compared with the control group.*

Table 6: of Cotton pellet induced granuloma in rats

Treatment	Dose (mg/kg	Dry weight of cotton pellets (mg)	% Inhibition
Control (Vehicle)	-	78.3± 1.41	-
Standard Drug (indomethacin)	10mg	21.2± 0.21***	72.92
MECL+ MEBC	250mg+ 250mg	39.1±0.31***	50.06
MECL+MEFB	250mg+ 250mg	42.3±0.73***	45.97
MEFB+MEBC	250mg+ 250mg	50.5±1.02***	35.50
MECl+MEFB+MEBC	200mg+ 200mg+ 100mg	53.75±0.84	31.35

*The values are expressed as mean± S.E.M , n=6 in each group, *** p≤0.001, ** p≤0.01, * p≤0.05 ns-non significant when compared with the control group.*

Statistical analysis

All data are presented as mean ± standard error mean (S.E.M.) Statistical differences between control and treated groups were tested by one-way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS AND DISCUSSION

The present results showed that methanol extracts of the selected plants administrated topically on croton oil-induced oedema in rat's ear skin caused different responses in inhibition of weight of ear. Most of methanol extracts *Curcuma longa, Lens culinaris, Bombax ceiba* and *Ficus bengalensis* were found to be active and reduced the weight of ears 0.044±0.006 µg, 0.062±0.005 µg, 0.051±0.010 µg and 0.049±0.012 µg respectively compared to control 0.072±0.010 µg and Positive control 0.035±0.008 µg. Especially methanol extracts *Curcuma longa* showed remarkable anti-inflammatory activities (Table 3).

Topical application of croton oil promoted an increase in the thickness of the ear. Upon application of the methanol extract of selected plants, croton oil-induced ear oedema and cellular migration in rats were both reduced effectively. Topically applied methanol extracts of plants resulted in an inhibition of croton oil induced ear oedema. Methanol extract of *Curcuma longa* showed a maximum inhibition of thickness of ear. In these tests, treatment with the methanol extract of *Curcuma longa, Lens culinaris, Bombax ceiba* and *Ficus bengalensis* showed significant inhibitions of ear thickness 82±09.11 µm, 115±16.12 µm, 91±12.12 µm and 89±11.01 µm respectively. (Table 4).

Our observations cannot specify the precise mechanism of the anti-inflammatory effect of our extracts of selected plants did not inhibit the oedema, weight of ear and thickness of ear in croton oil-induced oedema in rat.

Optical microscopic analysis of the rat's ears, 5 h after application of croton oil, revealed epidermal hyperplasia and marked infiltration of inflammatory cells associated with dilated blood vessels. These events were greatly reduced after topical application of methanolic extract as well as by the positive control.

The skin tissues were harvested 5 h after the applications of methanol extract and hematoxylin and eosin sections were then microscopically examined. Whereas vehicle treatment induces histological skin damages in rats, Methanol extract treatment induced mild acanthosis and thickness of the dermis with oedema and infiltration of mononuclear cells. In control rats, more severe hyperkeratosis, acanthosis, migration of inflammatory cells into the epidermis (exocytosis), and swelling of the dermis were observed. It is worth to note that vehicle-treated rats showed only but significant epidermal hyperplasia with nuclear swelling of keratinocytes and infiltration of mononuclear cells and eosinophils in the dermis .

In the pathology of acne topical inflammation was a major factor which aggravated acne production. So, topical anti-inflammatory activity of drug samples was studied.

In the present study, topical inflammation was induced by Croton oil. The main characteristics of Croton oil induced inflammation are of vascular permeability and vasodilation, which results in oedema and migration of inflammatory cells mainly neutrophils. The various parameters such as weight variation of ear, thickness variation of ear and histopathology of ears of all groups were studied. Our results clearly show that the ability of a methanol extracts to inhibit oedema in comparisons of the same plants. Indeed, we found that the methanol extract of *Curcuma longa* has shown better inhibition of oedema as compared to other extracts.

Histopathological evaluation of the treated ear of different groups showed variation in epidermis of skin. Toxin show a maximum increase in thickness of epidermis. Whereas the methanol extract of *Curcuma longa* was effective in controlling the effect of toxin and reduces epidermis thickness followed by methanol extracts of *Ficus bengalensis* and *Bombax ceiba.* The chronic anti-inflammatory effect of the MECL, MEBC and MEFB was assessed using cotton pellet induced granuloma method in albino rats illustrated in table 1.The MECL and MEFB at the dose 250 mg/kg showed maximum decrease in formation of granuloma tissue. The result indicates that MECL and MEFB produced a decrease in weight of granuloma 54.09% and 44.80% respectively in comparison to control. The potency of the extracts of was compared with the standard drug indomethacin (10mg/kg) which showed 67.75% inhibition in granuloma weight does dependently.

In carrageenan induced rat Paw oedema in rats the effect of MECL , MRBC and MEFB at dose 250mg/kg produced a significant effect against carrageenan induced inflammation after three hours of the administration. The potency of the extract was compared with the standard drug indomethacin (10mg/kg) MECL and MEFB tested dose showed highly significant ($p \leq 0.01$ to $p \leq 0.001$) decrease in rat paw oedema induced by carrageenan. The result MECL show 61.75% suppression where as MEFA at the same dose show 58.82% suppression of paw odema volume which is extremely significant comparable to standard drug indomethacin Table 2.

The result indicate a poly herbal extract combination of MECL + MEBC produced a decrease in the weight of the granuloma 64.63% and 53.65% respectively in comparism to control. The potency of the extract was compared with the standard drug indomethacin (10mg/kg) Table 5.

The result obtain MECL, MEBC 50.06% suppression where as other combination MECL and MEFB are 45.97% and MEFB and MEBC was 35.50% .And the combination of MECL, MEFB and MEBC was 31.35 % suppression of paw oedema volume which is extremely significant as comparable to standard drug indomethacin Table 6.

CONCLUSION

The findings of the present study indicate that the combined effects of *Curcuma longa, , Bombax ceiba* and *Ficus bengalensis is* being found to have beneficial effects against anti acne and other anti-inflammatory disorders.

Acknowledgement
This study is a part of the Ph.D thesis (Phytochemical and Pharmacological Screening of Poly-Herbal formulation for anti acne activity) of NIMS University, Institute of Pharmacy, Jaipur, Rajasthan.

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