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ORIGINAL ARTICLE **OPEN ACCESS**

Design, Development and Evaluation of Polyherbal Formulation for Anti-Allergic Activity Containing Some Indigenous Herbs: *Aegle marmelos, Annona squamosa, Acacia arabica, Butea monosperma*

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ABSTRACT
The present paper deals with the evaluation of anti-allergic activity and phytochemical study with polyherbal formulation. The therapeutic importance of the medicinal herbs like *Annona squamosa, Aegle marmelos, Acacia arabica, Butea monosperma*. This study involves the Anti-allergic activity on mice and phytochemical study of *Annona squamosa, Aegle marmelos, Acacia arabica, Butea monosperma*. preliminary screening of active constituents presented in the ethanolic extract of leaves of *Annona squamosa, Aegle marmelos* and bark of *Acacia arabica, Butea monosperma* plants. The extract are used to prepare the different formulation for there pharmacological activity study. In the Anti-allergic study, we have investigated the effect of different formulations against oxazolone-induced contact dermatitis in mice. Contact dermatitis was induced on the ear of balb/c mice by repeatedly applying Oxazolone. Dermatitis induced was observed with the help of sustained ear swelling, erythema and redness, epidermal hyperplasia, and marked infiltration of inflammatory cells which includes monocytes, granulocytes, and macrophages. In this study, we found that oxazolone-treated mice showed a marked increase in ear thickness, erythema, and redness but in the case of mice treated with different formulations significantly reduced ear thickness.
Keyword: Anti-allergic activity, poly-herbal, Oxazolone, sueroxide dismutase,glutathione.

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INTRODUCTION

In India different medicinal plants originated from the nature having different pharmacological activities. The present study deals with the medicinal herbs like *Annona squamosa, Aegle marmelos, Acacia arabica, Butea monosperma* are used to Anti-allergic activity study on animals[2-4]. In which the leaves of *Annona squamosa, Aegle marmelos* qnd bark of *Acacia arabica, Butea monosperma* are used to extraction process [5].The ethanol solvent was used to extraction process depending upon the large yield. The extract are used to prepare the different formulation for their pharmacological activity study. The dermatitis induced by applying oxazolone [1]. Then after inducing agent find out the changes in the animals and calculate the various factors like measurement of ear thickness, histopathology, tissue homogenization,estimation of superoxide dismutase, estimation of glutathione, cytokine determination etc. in different animals group with different treated different dosage formulations [6-11].

Material And Methods

The plants were selected on the basis of their pharmacological activities and their medicinal uses reported in the literature. The herbs (*Annona squamosa*, *Aegle marmelos, Acacia arabica, Butea monosperma*) were purchased from the Herbal garden of P.Wadhawani College Of Pharmacy,Yavatmal and authenticated by

Taxonomist in the department of Botany, Shri Shivaj i Science and Arts College, Chikhli. Dist. Buldana SGBAU University (MS) All other chemicals were of analytical grade and used without further purification.

Experimental Animals

Different formulations Balb/c mice (7-8 weeks old) were obtained from the institutional P. Wadhawani College of Pharmacy, Yavatmal animal house facility. Animals were housed in standard condition with temperature (25 ± 2^0c) and relative humidity (65%) as well as maintained light and dark cycles (12:12 hours). All animals were provided with standard diet and water *ad libitum*. The experimental protocols approved by the IAEC. Approval No. is 650/Po/Re/S2002/2022/CPCSEA/25.

Chemicals

Oxazolone was procured from Sigma Aldrich, India. Cytokine ELISA Ready SET-Go kits for IL-1β, IL-6, and TNF-α were procured from Bio vision. All reagents and chemicals utilized for experimentation were of the analytical grade and with high purity [1].

Preparation of extract

The powdered of *Annona squamosa*, *Aegle marmelos, Acacia arabica, Butea monosperma* were used for extraction. The powder is extracted in soxhlet apparatus with ethanol. The extraction procedure were carried out till a sufficient quantity of extract was obtained. The solvent was removed by distillation method [6] .

Formulation of dosage form:-

Procedure :- Carbopol 940 1gm and 1 gm measured quantity of extract was disperse in 80 ml of distilled water and mixed by stirring continuously in a magnetic stirrer at 800 rpm for 1 hour. Glycerol 5 ml added to the mixture under continuous stirring. The mixture was neutralized by drop-wise addition of 50% tri-ethanolamine (w/w). Mixing was continued until a transparent gel was formed. Three types of gel formulations were prepared. Formulation Ist gel containing 1.0% (w/w) Aegle marmelos leaf extract, Formulation IInd gel containing 1% (w/w) Annona squamosa leaf extract, Formulation IIIrd gel containing 1% (w/w) *Acacia Arabica* bark extract Formulation IVth gel containing 1% (w/w)*Butea monosperma* bark Formulation Vth gel containing 4% (w/w) Aegle *marmelos* leaf extracts, Annona *squamosa* leaf extract, Acacia *Arabica* bark extract and Butea *monosperma* bark extract (i.e. 1% of each extract).

Experimental Protocol

Forty Two mice were randomly allocated into Seven groups. Each group contains six mice and received the following treatment for 6 days.

G1-Disease control: 100µl of 2% oxazolone topically
G2-Standard: Treated with vehicle
G3- 100 µl of 2% oxazolone topically + formulation I
G4- 100 µl of 2% oxazolone topically + formulation II
G5- 100 µl of 2% oxazolone topically + formulation III
G6- 100 µl of 2% oxazolone topically + formulation IV
G7- 100 µl of 2% oxazolone topically + formulation V

Preparation of oxazolone

2% oxazolone was dissolved in acetone and olive oil in the proportion of 4:1 ratio and 100 µl was used for sensitization.

Induction of Dermatitis

The hairs from the abdomen of mice were removed by using an electrical caliper and confirmed the complete removal of skin hairs. The abdominal skin was sensitized by the application of 100µl of 2% oxazolone. Five days after this sensitization, the mice were again challenged with oxazolone (100µl) on the inner and outer surface of the right ear. The vehicle (Acetone) was applied to control group mice. The change in ear thickness was measured using vernier caliper every 24 hrs after each oxazolone challenge for the next 6 days [11].

Measurement of ear thickness

The ear thickness was measured using a vernier caliper every 24 hrs after the application of oxazolone on ear. The change in ear thickness was used to indicate the extent of inflammation in oxazolone induced[9].

Histopathology

Mice were anesthetized by using light ether anesthesia. Ear samples of each mouse were isolated after 24 hrs of the final application of oxazolone. Ear samples were fixed in formalin (10%), paraffinized, and 4 mm sections were taken, finally stained with Hematoxylin and Eosin (HE) for examining for pathological changes and cell infiltration under the microscope [12].

Tissue homogenization

At the end of the experimental treatment protocol (on day 6) animals were sacrificed by a high dose of anesthesia. The animal ear from each group was cut using fine scissors and samples were collected and kept in liquid nitrogen till the use. The ears were homogenized in phosphate buffer saline (PBS) (pH-7.4)

and stored at -20˚C for further assays. The ear tissue homogenate was used to assay the inflammatory cytokine, GSH.

Estimation of Superoxide Dismutase

The SOD levels from the ear tissue homogenate were estimated by using the formerly reported method. The assay procedure was as defined in brief, the reaction mixture contains 0.4 ml of ear tissue homogenate, diethylenetriamine penta acetic acid (1 mM), 0.5 ml of pyrogallol (0.2 mM) in 50mM Tris buffer (pH 8.5). The reaction mixture incubed different formulations at room temperature (RT) for 1.5 hrs. Finally, the absorbance different formulations was taken at 420 nm [13].

Estimation of Glutathione

The reagents of H_2O_2, 1mM GSH (glutathione), 0.2mM NADPH, and the tissue homogenate were added to 0.1 M Tris–HCl buffer solution (pH 7.2) and reacted at 25 ^{0}C for 5 min. NADPH consumed by the reduction of the oxidized form of glutathione was determined by measuring absorbance different formulations at 340 nm and glutathione (GSH) activity was calculated. Enzyme activity was quoted as the units of NADPH oxidized nmol/1 mg protein/min.

Cytokine determination

The ear tissue homogenate from all the group animals was used for the estimation of inflammatory cytokines (TNF-α, IL-6, and IL-1β) using ELISA assays. The assay was performed according to the manufacturer provided protocol. Final values are calculated from the standard curve [22].

Statistical analysis

Data were expressed as mean ± SEM and analyzed with one way ANOVA followed by Bonferroni's multiple comparison tests. P-value is less than 0.05 is considered statistically significant. ***P < 0.001; as compared to oxazolone group and ###P < 0.001 as compared to standard mice.

RESULT

Effect of the treatments on clinical features and ear thickness

Oxazolone sensitized mice showed progressive symptoms of erythema, swelling, and redness of skin as compared to the standard mice. In case of formulation treated mice showed clinical improvements in erythema, swelling and redness shown in figure 1. In oxazolone challenged group shows increased thickness, erythema and redness from day 2 to throughout experimental days as compared to the standard group. formulation V shows suppressed ear thickness, erythema and redness as compared to the oxazolone treated group.

Effect on proinflammatory cytokine

We investigated the effects of formulation on pro-inflammatory cytokines in-ear. There were significant increases in the protein levels of TNF-α, IL-6 in the ear of dermatic mice, as compared with the standard group (P < 0.001). However, formulation treated significantly reduced the level of TNF-α, IL-6, and IL-1β compared to oxazolone treated control (P<0.05). The effect of formulation 5 treated shows a most prominent effect on cytokine levels.

Effect of treatment on oxidative stress

We used the ear homogenate to estimate the extent oxidative stress in oxazolone induced increase in oxidative stress. We found that oxazolone sensitized mice shows decrease in the GSH and SOD in ear tissue homogenates. The mice treated with formulation V showed an increase in the content of GSH and SOD.

Effect on histology of ear

In the macroscopic examination of mice ear with oxazolone sensitized showed marked and significant inflammatory changes, with epidermal thickening, dermal thickening, epidermal hyperplasia, and strong dermal infiltration of inflammatory cell as compared to standard mice. formulation I and II treated mice showed more effective.

Fig.1*Aegle marmelos*.

Fig.2*Annona squamosa*

Fig.*3Acacia Arabica*

Fig.4*Butea monosperma*

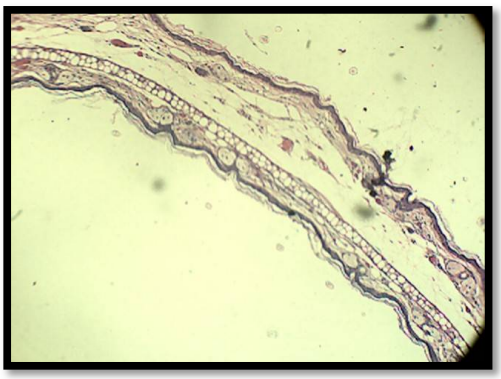


Fig.5 Group1.Disease control

Fig.6 Group2 Standard (Treated with Vehicle)

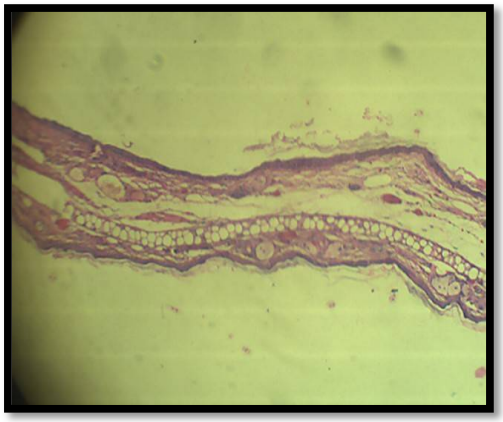


Fig7. Group3 (Oxazolone + Formulation I)

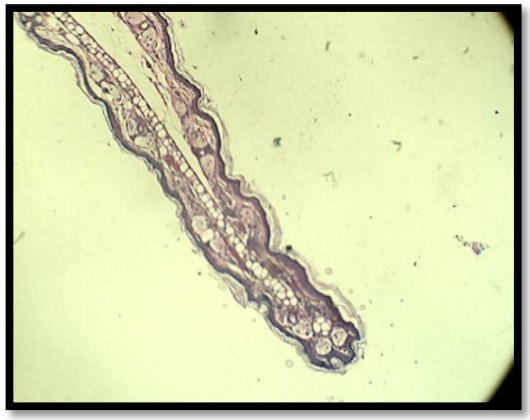


Fig8.Group 4 (Oxazolone + Formulation II)

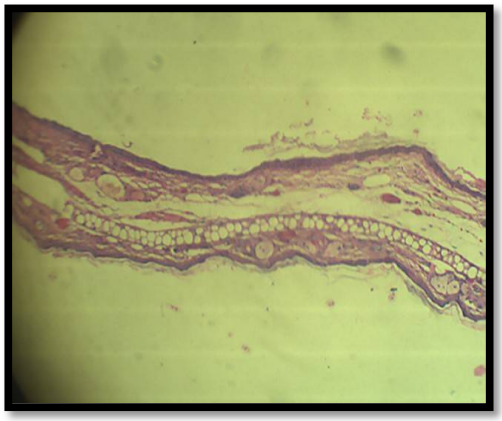


Fig.9 Group5 (Oxazolone + Formulation III)

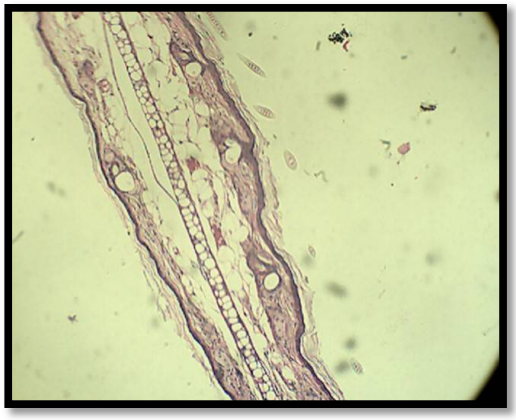


Fig.10 Group6 (Oxazolone + Formulation IV)

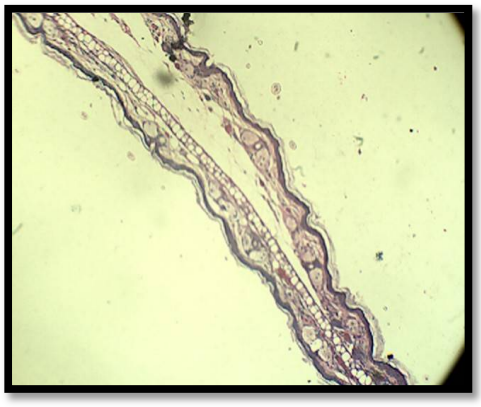


Fig.11 Group7 (Oxazolone + Formulation V)

Table 1. Effect on proinflammatory cytokine (TNF-α).

Mean	59.12	36.31	51.33	49.74	42.76	37.61	35.54
Std. Deviation	7.807	1.643	4.793	1.106	2.086	1.723	2.335
Std. Error of Mean	3.904	0.8216	2.396	0.5528	1.043	0.8613	1.168

Table 2. Effect on proinflammatory cytokine (IL-6)

Mean	97.33	63.07	79.78	81.37	86.77	68.16	63.20
Std. Deviation	6.862	6.131	4.188	5.484	4.059	2.353	2.933
Std. Error of Mean	3.431	3.066	2.094	2.742	2.030	1.177	1.467

Table 3. Effect on proinflammatory cytokine (IL-1B)

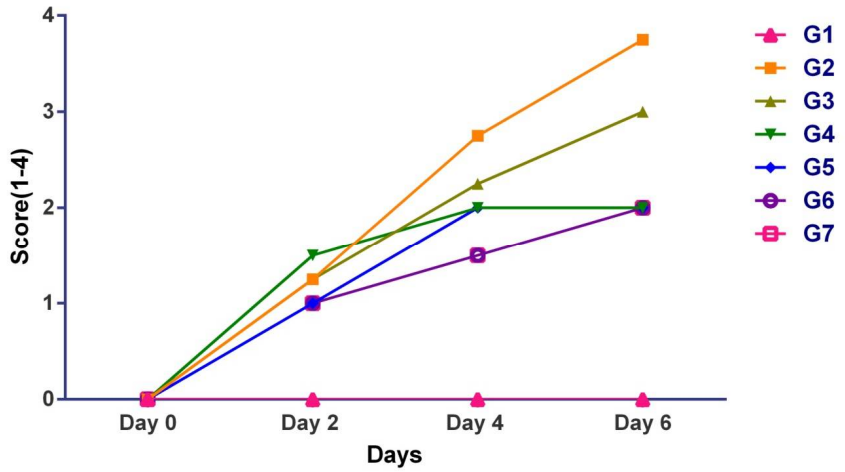
Mean	32.98	16.28	28.84	28.78	26.08	16.27	15.50
Std. Deviation	2.777	3.270	2.384	1.314	1.742	0.8222	2.217
Std. Error of Mean	1.389	1.635	1.192	0.6569	0.8712	0.4111	1.109

Table 4. Effect of treatment on oxidative stress (Superoxide Dismutase)

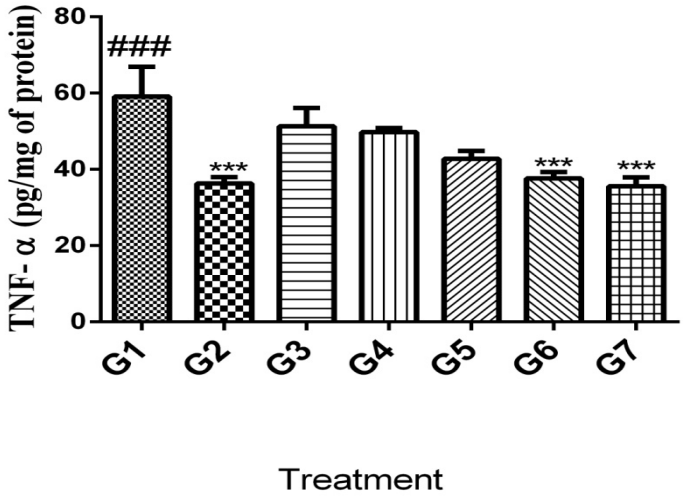
Mean	1.293	2.733	1.553	1.635	1.558	1.838	1.973
Std. Deviation	0.1578	0.1758	0.1644	0.3083	0.2490	0.06076	0.1226
Std. Error of Mean	0.07889	0.08788	0.08220	0.1541	0.1245	0.03038	0.06129

Table 5. Effect of treatment on oxidative stress (Glutathione)

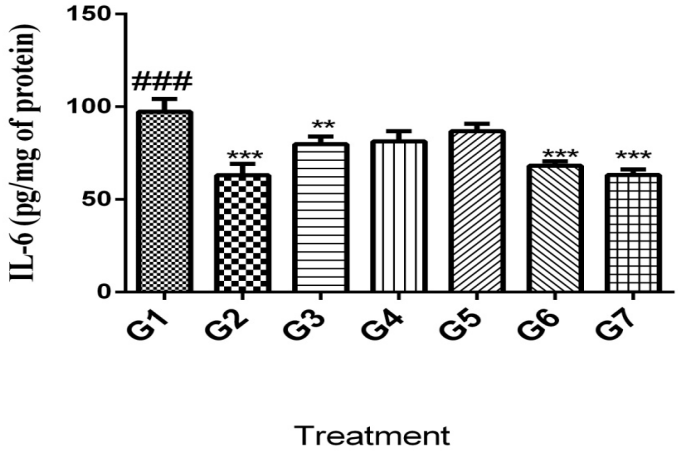
Mean	0.9893	1.983	1.158	1.120	1.163	1.183	1.568
Std. Deviation	0.04158	0.1588	0.1307	0.06055	0.05679	0.06652	0.1746
Std. Error of Mean	0.02079	0.07941	0.06537	0.03028	0.02839	0.03326	0.08731



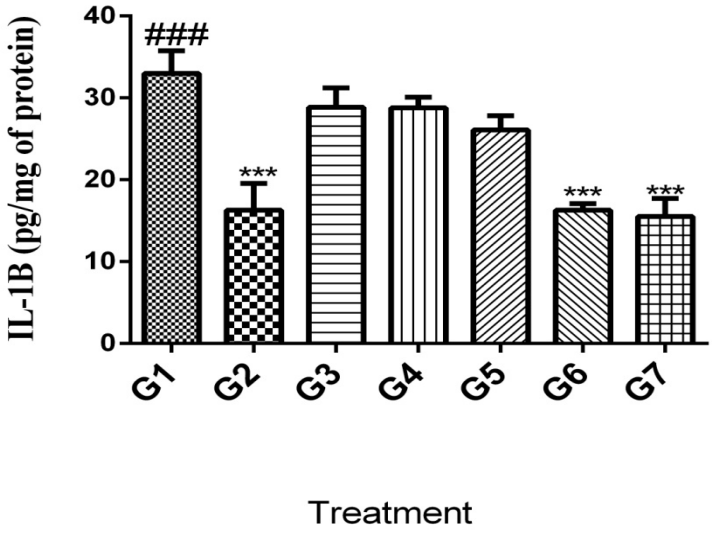
Graphical presentation 1. Effect of the treatments on clinical features and ear thickness



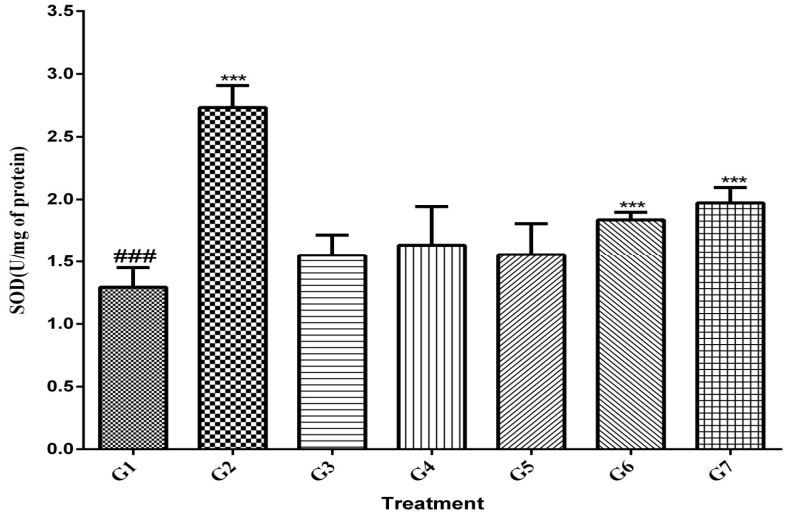
Graphical presentation 2. Effect on Effect on proinflammatory cytokine **(**TNF-α).



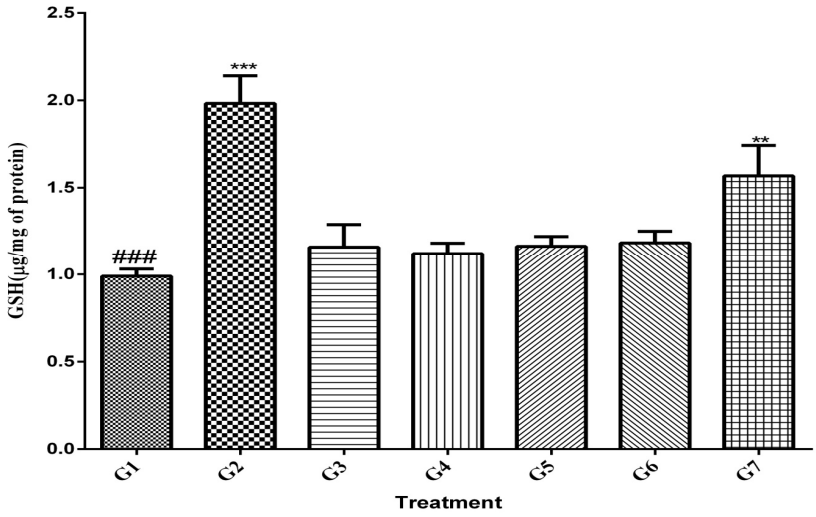
Graphical presentation 3. Effect on Effect on proinflammatory cytokine (IL-6)



Graphical presentation 4. Effect on Effect on proinflammatory cytokine (1L-1B).



Graphical presentation 5. Effect of treatment on oxidative stress (Superoxide Dismutase)



Graphical presentation 6. Effect of treatment on oxidative stress (Glutathione).

DISCUSSION

In the present study, we have investigated the effect of different formulations against oxazolone-induced contact dermatitis in mice. Contact dermatitis was induced on the ear of Different formulations on balb/c mice by repeatedly applying Oxazolone. Dermatitis induced was observed with the help of sustained ear swelling, erythema and redness, epidermal hyperplasia, and marked infiltration of inflammatory cells which includes monocytes, granulocytes, and macrophages [14-16]. In this study, we found that oxazolone-treated mice showed a marked increase in ear thickness, erythema, and redness but in the case of mice treated with different formulations significantly reduced ear thickness.

Pro-inflammatory cytokine-like TNF-α, IL-6, IL-1β plays a key role in the development of inflammation in dermatitis. Recent studies have proved that the cytokines have critical for the pathogenesis of dermatitis in humans, play an important role in mice skin inflammation because the expression of cytokines is increased in the skin lesions. Many reported studies proved that oxazolone challenged mice to show an elevated level of pro-inflammatory cytokines like TNF-α, IL-6, IL-1β. In the present study, we found a similar effect and on treated mice lowered the level of cytokines. In this study, we found that mice challenged with oxazolone show an increase in cytokine level and different formulations treated mice prevented this effect. In dermatitis increase in oxidative stress was found in oxazolone-challenged mice this effect similar to the reported articles and different formulations -treated mice show the revert effect [17-20]. The histopathological examination of mice with oxazolone sensitized shows marked and significant inflammatory changes like an increase in epidermal thickness, dermal thickening, epidermal hyperplasia, and strong dermal infiltration of the inflammatory cell. A similar result found in oxazolone challenged mice and different formulations treated mice showed a protective effect in histopathological changes.

CONCLUSION

The present study, we conclude that the Anti-allergic study in mice. Having the leaves and bark extracts which are used to prepare the gel formulation. with different gel formulations showed the anti-dermatic activity by a decrease in ear thickness, protection from an increase in pro-inflammatory cytokine level, maintain oxidative stress, and preventing the pathological changes in ear histology.

Data availability statement

All data analyzed during this study are included in this article.

Funding

For the submission this study, does not include any research funding.

Competing interests

The authors declare no conflict of interest with this research.

Ethical approval

Animals ware used in this study, ethical approval No. is 650/Po/Re/S2002/2022/CPCSEA/25. Different Balb/c mice (7-8 weeks old) were obtained from the institutional P. Wadhawani College of Pharmacy, Yavatmal animal house facility.

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