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BIOLOGICAL EVALUATION OF SELECTED PHYTOCHEMICALS FOR THEIR ANTI-ACNE PROPERTIES BY CHEMICAL INDUCED ACNE VULGARIS IN RODENTS

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ABSTRACT

The present work is to screen the selected phytochemicals for alleviate chemical induced acne in mice. The treatment for acne with synthetic drugs have various drawbacks, usage may lead to development of resistant towards these drugs. Hence plants derived compounds are required to overcome the above drawbacks and treat the acne. Present study was aimed to evaluate anti acne potential of selected phytochemicals on the primary clinical lesion, microcomedone, which are the precise, almost all other acne lesions by applying topically on skin. Animals of either sex were collected from the animal house of Chalapathi Institute of Pharmaceutical sciences were randomized into 10 groups consisting of five animals/ group. Benzalkonium chloride used for inducing acne. The test compounds (2% geraniol, 2% hesperidine 2% ellagic acid and 2%liquorice) showed that decreased the inflammation in mice ear. On the10th day there was a significant decrease ($p<0.0001$) in inflammation (0.66±0.1503, 0.32±0.0734, 0.32±0.0734) with respective to the disease control. The test compounds at 2% showed significant reduction in the overall damage caused by acne induced by chemical contact and reduced the proliferation of inflammatory cell into the ear region.

Keywords: Acne, Benzalkonium chloride, Ellagic acid, Hesperidine, and Geraniol

1. INTRODUCTION

Acne vulgaris, a chronic inflammatory disorder in adolescent consist of the pilosebaceous follicles, characterized by comedones, papules, cyst, and nodules often scars, chiefly on face, neck etc. The inflamed glands caused by stress, hereditary factors, hormones, drugs and bacteria. Cause of acne includes the action of sebum synthesized and secreted by sebaceous glands, due over production of hormones in both girl and boy during puberty, exposure of skin to chemicals. Hormonal change related to pregnancy, stopping birth control pills, stress, skin irritation and Heredity **[1]**. There are four main interacting factors in the pathogenesis of acne vulgar a) Increased sebum production, b) Microbial flora changes, c) Abnormal keratinization, d) Inflammation. The aim is to eliminate the primary clinical lesion, microcomedone, which is the precise almost all other acne lesions. There are a lot of topical or systemic agents for this purpose **[2]**.The medication have several adverse effects like birth defects, erythema, photosensitivity, allergic dermatitis, excessive skin irritation, urinary problem, joint and muscle pain, headache, depression etc. Many remedies have been employed to treat acne from long period. Most of the remedies were taken from plants and proved to be useful, scientifically established except for a few plants and some proprietary composite herbal drugs **[3]**. Topical retinoid are important tools in the management of acne because they act against comedones and microcomedones, and have direct anti-inflammatory effects. The retinoids approved for acne treatment include tretinoin and isotretinoin as well as the synthetic third-generation polyaromatic retinoids adapalene and tazar-otene. Although the minimal systemic availability of topical retinoid creams has been confirmed, teratogenicity seems to be the most worrisome effect of the retinoids since. It was first observed in rat experiments **[4]**. To overcome the potential risk and adverse effects of antibiotic resistance from prescription medications, traditional herbal medicines have been extensively studied as alternative treatments for many diseases. In this, the potential use of phytochemicals as a basis for new skin-care cosmetics has been emphasized recently. However, most of the natural components(isolated from several different plant species)have antimicrobial activity considerably less potent than that of synthetic drugs **[5]**.The cosmetics available in the market are not reasonable for everyone thus an effort has been made to study their properties for anti acne activity and to incorporate these extracts in the formulations. The product may be cost

effective. This has given rise to stimulation in the search for investigating natural resources showing anti-acne activity **[6-8]**.

Flavonoids, tannis, glycosides, and Monoterpenes, have been considered has effective for the treatment various disorders in humans. All four selected compounds have been widely used as active ingredients in many pharmaceutical products due to their analgesic, anti-inflammatory, anti-pain and wound healing properties **[9]**.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

Hesperidine , ellagic acid, liquorice , geraniol and benzalkonium chloride were purchased from sigma Aldrich and other chemicals and solvents used are analytical grade

2.2 Experimental animals

60 mice were collected from the animal house of Chalapathi Institute of Pharmaceutical sciences were randomized into 10 groups consisting of six animals/ group. Group 1 served as diseased control. Acne was induced to the animals of group 1 to 10.Group 1 animals did not receive any treatment with the phytochemical and hence served as the disease control. Group 2 animals were treated with simple ointment served as vehicle control; group 3 is treated with dexamethasone at the dose of 0.1% by applying on to the upper surface of the ear. Animals of groups 3 to 10 were applied 1% and 2% of the ointment prepared by using selected phytochemicals respectively. The protocol of the study was approved by Institutional Animal Ethics Committee (IAEC) with approval number (No: 15/IAEC/CLPT/2018-19; Dt: 05/01/2019).

2.3 Procedure for preparation of ointment: [10,11]

The ointment was prepared by incorporating the phytochemicals (1% and 2% w/w) in the ointment base which was prepared by using following ingredients.

Hard paraffin	0.12gms
White bees wax	0.18gms
Cetostearyl alcohol	0.3gms
White soft paraffin	5.4gms
Test Drug	0.1gm

2.4 Qualitative analysis of phytochemicals

I. Flavonoids (Hesperidine)

- Small quantity of hesperidine was dissolved in aqueous sodium hydroxide solution. Appearance of yellow colour indicates presence of flavonoids.
- **Shinoda test**: Small quantity of sample was dissolved in alcohol. To this few pieces of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour indicates the presence of flavonoids.

II. Tannins (Ellagic acid)

- **Ferric chloride test**: Small quantity of Ellagic acid was taken in test tube and it was boiled with distilled water and then

filtered. To the filtrate, few drops of 5% ferric chloride solution were added. Violet colour is observed it indicated the presence of tannins.

- Ellagic acid is taken in to test tube to this 1%solution of gelatin containing 10%soium chloride. White colour precipitate indicate presence of tannins

III. Glycosides (Liquorice)

- **Foam test (saponin glycosides):** 10 mg of liquorice powder was taken in 50ml measuring cylinder, added water up to 25ml of the measuring cylinder and mark it as point 'A'. Shake vigorously for 5 minutes. Allowed it to stand for 30minutes. Now marked the final volume as point 'B' and measured the distance from 'A to B'. It shows the formation of Foam with increasing volume. This indicates presence of "Saponin glycoside."

IV. Aromatic oils (Geraniol):

- **Terpenoid test (Salkowski test):** 5 ml of geraniol was mixed with 2 ml of chloroform and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was observed; it showed positive results for the presence of terpenoids **[12, 13]**.

2.5 Parameters observed: Ear thickness, % inhibition of inflammation and histo-pathology of animal skin

2.6 Pharmacologicl screening:

- The animals were divided randomly in to 10 groups at which each group containing five mice having same age and weight.
- Those 10 groups treated as group-1 (Disease control), group-2, (standard), group-3 (1% hesperidine), group-4 (2% hesperidine), group-5 (1% Ellagic acid), group-6 (2% Ellagic acid), group-7 (1% Liquorice), group-8 (2% Liquorice), group-9 (1% Geraniol) and group-10 (2% Geraniol).
- Acne was induced by repeated application of benzalkonium chloride to the ear skin of mice through topically.
- After the visual confirmation of parameters of Acne, mice were treated with test samples.
- Both the standard (dexamethasone) and test were applied to the skin up to 3 weeks. After completion of treatment, mice were euthanized, ear skin were excised, fixed in formalin solution and prepared for histological studies

3. RESULTS

All compounds were tested for the acute dermal toxicity studies. All test compounds were free from allergy itching and rashes on the skin. There is no change in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern **[14]**.

The acne- was induced on the ears of mice by topical application of benzalkonium chloride. Ear thickness was measured as an index of inflammatory strength, using a micro indicator once every others day for the first week, then every other day until the 25th day. Thus the **(Table 1)** reveals that the maximum inflammation on ear was on 7th day in all groups. In test and standard treated group there was a sudden decrease in inflammation from 10th day which was constant till the 20th day and around 25th day the inflammation is reduced, came to normal. The result of the test compounds at two concentrations was comparable with standard. The data resulted from anti-acne effect of, 2% hesperidins 2% geraniol, 2%liquorice and 2% ellagic acid showed that decreased the inflammation in mice ear. On the 15th day there was a significant decrease ($p<0.0001$) in inflammation (0.66±0.1503, 0.98±0.1959, 0.32±0.0734, 0.54±0.1029) by respectively. In 1% ointment was significant ($p<0.05$) decrease in inflammation (0.82±0.1067, 0.54±0.1029, 0.68±0.0860 &0.52±0.12).

Histological changes

In histopathology of ear skin was found that accumulation of neutrophils on inflammatory lesions site with subsequent rupture of the follicle and formation of a pustule in the dermis and the transmigration of lymphocytes into the wall of the follicle associated with increasing spongiosis of the follicular epithelium **(Figure 3)**. During 24-72 hours, the accumulation of neutrophils within the follicle led to its distension and subsequent rupture. There was a localized loss of the granular layer in the region of the eventual rupture. This shows the difference of normal and acne- induced ear section. The treated ear section showed decreased infiltration of neutrophils, which was similar in standard drug treated **(Figure 3)**. The histopathology study supports the results shown in **(Table No. 2)** decreased inflammation in animals treated with phytochemicals from 15th to 25th day.

Table 1: Effect of phytochemicals on ear thickness (swelling) of chemical induced acne

Treatment	Ear thickness					
	Day 3	Day 7	Day 10	Day 15	Day 20	Day 25
Disease Control	1.36±0.1805	1.38±0.1772	1.6±0.1140	1.72±0.1428	1.72±0.2289	2.24±0.1152
Vehicle Control	0.3±0.0316	0.3±0.0316	0.3±0.0316	0.3±0.0316	0.3±0.0316	0.72±0.4211
Standard	0.76±0.0678	1.18±0.0860	1.14±0.1077****	1.04±0.0927****	0.92±0.0663****	0.58±0.0663****
Hesperidine 1%	1.34±0.0927	1.54±0.1503	1.2±0.1303**	0.82±0.1067**	0.58±0.037**	0.56±0.0871**
Hesperidine 2%	1.66±0.1749	1.74±0.1029	1±0.1732****	0.66±0.1503****	0.56±0.0871****	0.4±0.0447****
Ellagic acid 1%	0.94±0.2249	1.6±0.1224	0.82±0.1392**	0.52±0.12**	0.46±0.0812**	0.42±0.058**
Ellagic acid 2%	0.86±0.2803	1.4±0.1414	0.82±0.1772****	0.54±0.1029****	0.48±0.08****	0.4±0.0447****
Liquorice1%	1.2±0.2880	1.16±0.1029	0.96±0.1029**	0.68±0.0860**	0.62±0.0663**	0.42±0.0374**
Liquorice2%	0.72±0.1113	1.7±0.1303	0.54±0.0748****	0.32±0.0734****	0.34±0.04****	0.32±0.02****
Geraniol1%	1.04±0.1363	1.44±0.1536	0.76±0.1077**	0.54±0.1029**	0.6±0.063**	0.5±0.0447**
Geraniol2%	1.18±0.3215	1.46±0.1630	1.32±0.18****	0.98±0.1959****	0.58±0.08****	0.4±0.0447****

Values are expressed as mean ± SEM (n=5); Data are analyzed by Two-way ANOVA followed by Tukey's multiple comparisons test, ****P<0.0001 *vs* disease control

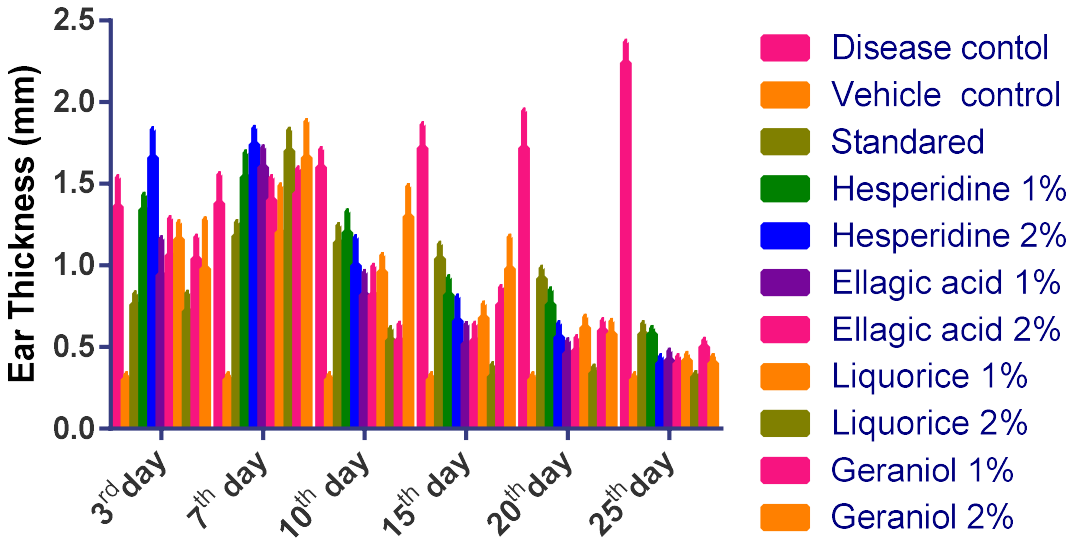

Figure No.1: Effect of Phytochemicals on ear thickness of mice treated with banzalkonium chloride

Table 2: Effect of Phytochemicals on ear inflammation induced by chemical in at various days

Groups	Percentage of ear inflammation					
	Day 3	Day 7	Day 10	Day 15	Day 20	Day 25
Disease control	60.7	61.6	71.4	76.7	76.7	100
Vehicle control	13.3	13.3	13.3	13.30	13.30	13.30
Standard	33.9	52.6	50.8	46.4**	41.0**	25.8**
Hesperidine1%	59.8	68.7	53.5	36.6**	25.8**	25**
Hesperidine2%	73.2	77.6	44.6	29.4**	25**	17.8**
Ellagic acid1%	41.9	71.4	36.6	23.2**	20.5**	18.7**
Ellagic acid2%	38.3	62.5	36.6	24.1**	21.4**	17.8**
Liquorice1%	53.5	51.7	42.8	30.3**	27.6**	18.7**
Liquorice2%	32.1	75.8	24.1	14.2**	15.1**	14.2**
Geraniol 1%	46.4	64.2	33.9	24.1**	26.7**	22.3**
Geraniol 2%	52.6	65.1	58.9	43.2**	25.8**	17.8**

Values are expressed as mean ± SEM (n=5); Data are analyzed by One -way ANOVA, **P<0.05 *vs* disease control

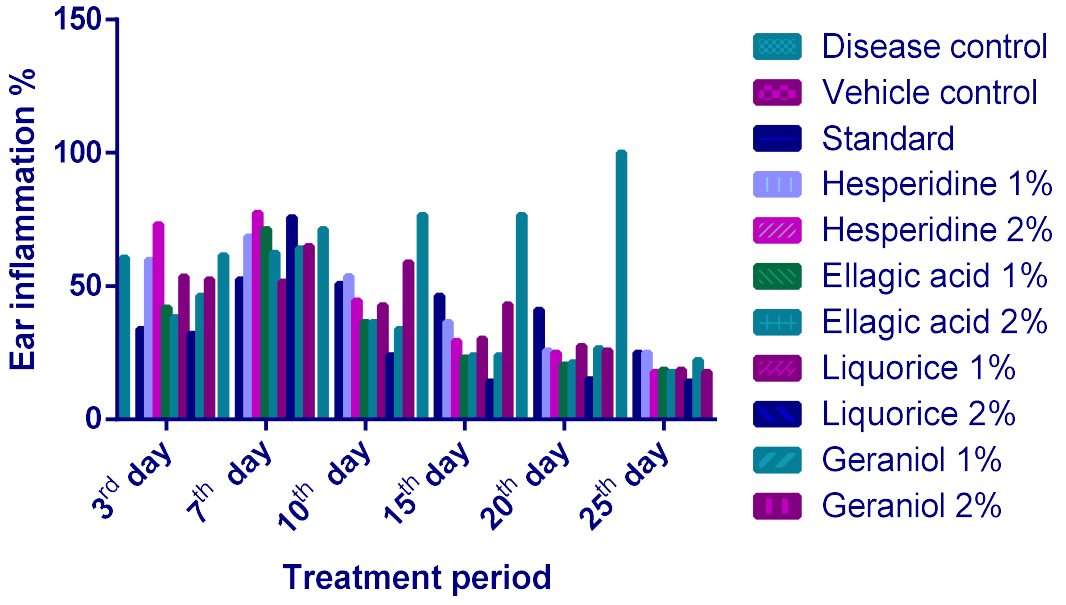

Figure 2: Effect of Phytochemicals on chemical induced acne on mice ear (benzalkonium chloride) from 3 rd day to 25th day

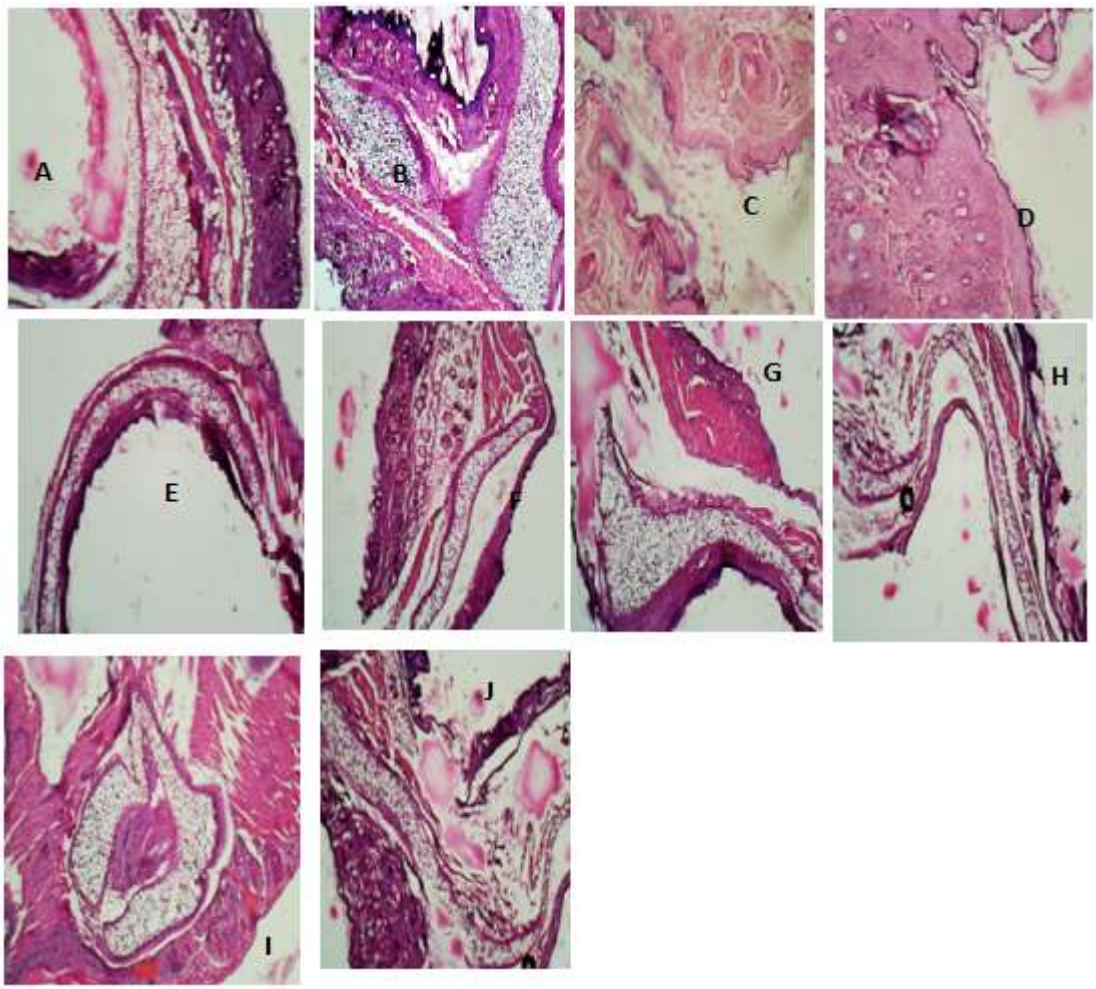

Figure 3: Histological changes in epidermis of ear skin with characterised by decreased proliferation of inflammatory mediators in the epidermis of ear skin.(a)Disease control ,(b)Standard(c) 1% hesperidine (d) 2%hesperidine (e) 1%ellagic acid (f) 2%ellagic acid (g) 1%liquorice (h) 2% liquorice (i) 1% geraniol (j) 2% geraniol

4. DISCUSSION

Acne vulgaris is a chronic inflammatory disease results in the formation of inflamed and/or no inflamed eruptions due to, chemical contact to the skin which are grow in the sebaceous region. Various antibiotics like tetracycline, Clindamycin, and erythromycin etc and other drugs like benzoyl peroxide are used for acne treatment. The various drawbacks of synthetic drugs are different side effects and resistant developed towards these drugs. Phytochemicals is required to overcome the above drawbacks and treat the acne. So in the present study four different phytoconstituents from different family (hesperidine, ellagic acid, liquorice and geraniol) were selected for evaluation of the anti acne activity. The preliminary qualitative analysis was carried out to estimate the purity of chemicals. As per literature review various phytocontituents like resinins, alkaloids, triterpenoids and flavonoids are proved to possess antibacterial activity. The acne like inflammatory activity was carried out by

measuring the ear thickness and histopathological studies of the ear. 1% ointment preparation of all the four phytoconstituents showed mild reduction in the ear thickness. It seems that the increased ear thickness and decrease inflammation caused due to various biochemicals, viz. various kinins, histamine and 5- HT is reduced compare to the disease control. Hesperidine, ellagic acid, liquorice and geraniol are the active anti-inflammatory constituents. The 2% ointment of all four phytoconstituents showed significant reduction in the overall damage caused due to benzyalkonium chloride, which can be seen in the histopathological studies. The literature review shows that inflammation is caused due to ROS (Reactive Oxygen Species). The four phytoconstituents were proved potent anti-oxidants they showed significant anti-acne properties which is supported by the histopathological studies.

5. CONCLUSION

The presented data indicate that the topical administration of 1% and 2 % ointment prepared by hesperidine, ellagic acid, liquorice and geraniol decreased inflammation, decreased the ear thickness and volume of sebum. The 2% ointment of phytochemicals has potent anti-acne activity, which can be useful for treatment of acne.

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