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SCREENING OF BIOACTIVE PHYTOCHEMICALS FOR THEIR ANTI-ALLERGIC ACTIVITY ON GUINEA PIGS AGAINST NON-IMMUNOLOGICAL CONTACT URTICARIA

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Keywords:

Urticaria, Hesperidin, Ellagic acid, Benzoic acid and Geraniol

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ABSTRACT: Pharmacological screening of selective phytochemicals for common skin allergic disorder, which is defined by the occurrence of itchy and even painful wheels, angioedema or erthyma (Urticaria). The main cause of allergic reactions is chemicals, certain foods, insect stings, which often lead to the cause of histamine release, then the tiny blood vessels (capillaries) leak fluid. For induction of urticaria (BA & CA) were selected; they cause the NICU on animal skin whenever they contact the skin. Healthy adult guinea pigs weighing 300-400 gm, were selected for the present study, totally 5 groups each contains five guinea pigs. Those groups were considered as group-1 (Disease control), group-2 (standard), group-3 (Vehicle control), group-4 (test-1) and group 5 (test-2). Parameters such as ear thickness, redness, itching, swelling and time taken for reducing swelling was recorded. Topical application of Benzoic acid and Cinnamon acid to all groups, those animals produced signs of urticaria, which is characterized by redness, itching, swelling. A dose dependent increase in ear thickness was observed in disease control. Topical treatment of phytochemicals reduces redness on guinea pig ears, ear thickness; it is significant reduced with 1% w/w ointment treated group (0.27±0.01****, 0.318±0.015****, 0.266±0.015****) when compared with the disease control group. 2% w/w ointment treated group also produced the significant effect with high reduction of ear thickness which is similar to that of standard group (0.19±0.008****, 0.214±0.009****, 0.154±0.014****). Ear thickness ****P<0.001 *vs.* disease control. Based on the results, it was concluded that selected phytochemicals are suitable for treating NICU without complications.

INTRODUCTION: The skin is the largest organ of our body, accounting for about 15% of total body weight. It is continuous with the mucous membranes lining the body surface. It is a complex organ; an average square inch of skin consists of 650 sweat glands, 20 blood vessels, and more than 1000 nerve endings [1]. Urticaria is a skin rash triggered by a reaction to food, medicine, or other irritants. It is the dermal edema resulting from vascular dilation and leakage of fluid into the skin in response to molecules released from mast cells. The major preformed mediator histamine produces a prototypic, short-lived urticarial [2]. Acute Urticaria is most often a benign self-limited skin disease.

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It occurs independently, but it may contribute to the more serious clinical manifestations of anaphylaxis [3]. First-line therapy consists of second-generation non-sedating anti-histamines, which are directed upon symptomatic relief of urticaria by antagonizing the specific actions of H_1 receptor-mediated histamine actions upon endothelial cells (the wheal) and on the sensory nerves (pruritus). Second-line therapy, corticosteroids –mostly methylprednisolone along with levocitrizine 5mg is used. Cyclosporine, omalizumab, is a humanized monoclonal IgG antibody against IgE, with low immunogenicity [4]. Treatment of these drugs may cause unwanted effects and develop tolerance early.

Hesperidin, ellagic acid, glycyrrhizic acid, and geraniol are well-known phytochemicals that have a therapeutical potency to treat various infections and diseases. However, there is no scientific proof or publications support the anti-allergic activity of these compounds in Non-Immunological Contact Urticaria [5]. Experimentally the NICU triggered by the topical application of haptens, such as benzoic acid (BA), sorbic acid (SA), cinnamic acid (CA), cinnamaldehyde (CAL), methyl nicotinate (MN), and dimethyl sulfoxide (DMSO).

In the present study, we made an attempt to evaluate the therapeutic efficacy of selected phytochemicals on NICU- induced by benzoic acid (BA) and cinnamic acid (CA), in guinea pigs [6].

MATERIALS AND METHODS:

Experimental Animals: Healthy adult guinea pigs weighing 300-400 gm were selected for the present study which is kept under optimal temperature with proper diet. The experimental procedure was approved by IAEC (23/IAEC/CLPT/2018-2019) dt:05-01-2019 of Chalapathi Institute of Pharmaceutical Sciences. Totally 5 groups each contain five guinea pigs

Collection of Phytochemicals: All phytochemicals (hesperidin, ellagic acid, glycyrrhizic acid, and geraniol) and bezoic acid, cinnamic acid was purchased from National scientific products, Guntur.

Preparation of Simple Ointment: Simple ointment was prepared by using the below ingredients. The test, standard, inducing agents are mixed with that to produce required concentration of ointments.

S. no.	Ingredients	Quantities Required
1	Hard Paraffin	0.3g
2	White Soft Paraffin	9 g
3	Cetostearyl Alcohol	0.5 g
4	Bees Wax	0.2 g

Preliminary Phytochemical Analysis:

Flavonoids (Hesperidin):

- Small quantity of hesperidin was dissolved in an aqueous sodium hydroxide solution. The appearance of a yellow color indicates the presence of flavonoids.
- Shinoda test: Small quantity of samples was dissolved in alcohol. To this few pieces of magnesium followed by concentrated hydrochloric acid were added drop-wise and heated. The appearance of magenta color indicates the presence of flavonoids.

Tannins (Ellagic acid):

- Ferric chloride test: Small quantity of Ellagic acid was taken in a test tube, and it was boiled with distilled water and then filtered. To the filtrate, a few drops of 5% ferric chloride solution were added. Violet color 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 color precipitated indicate the presence of tannins

Glycosides (Glycyrrhizic acid):

- Foam test (saponin glycosides): 10 mg of glycyrrhizic acid powder was taken in 50 ml measuring cylinder, added water up to 25ml of the measuring cylinder, and mark it as point ‘A’. Shake vigorously for 5 min allowed it to stand for 30 min. 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 the presence of “Saponin glycoside.”

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 [7].

Experimental Design for Non-immunological Contact Urticaria using Benzoic Acid:

- 5 groups each contain five guinea pigs selected with the same age and weights are kept in individual cages.
- Those groups were considered as Group-1 (Disease control), Group-2 (standard), Group-3 (Vehicle control), Group-4 (1% w/w of test) and Group 5 (2% w/w of test).
- All those groups of guinea pigs were treated with benzoic acid on both of the ears for inducing NICU.
- After that, animals were treated with respective standard and test compounds.
- Hesperidin, Ellagic acid, glycyrrhizic acid, and geraniol with a concentration of 1% and 2% to the respective group of animals
- Parameters such as ear thickness, redness, itching, time taken for reducing swelling were recorded.

Experimental Design for Non-immunological Contact Urticaria using Cinnamic Acid:

- 5 groups each contain five guinea pigs were selected which are having same age and weights are kept in individual cages.
- Those groups were considered as Group-1 (Disease control), Group-2 (standard), Group-3 (Vehicle control), Group-4 (1% w/w of test) and Group 5 (2%w/w of test).
- All those groups of guinea pigs were treated with Cinnamic acid on both of the ears for inducing NICU.
- After that, animals were treated with respective drugs, standard, and test compounds.
- Hesperidin, ellagic acid, glycyrrhizic acid and geraniol with concentration of 1% and 2% to respective group of animals.
- Parameters such as ear thickness, redness, itching, time for reducing swelling was recorded [8].

RESULTS:

Phytochemical Analysis: Preliminary qualitative phytochemical analysis evidenced the presence of corresponding active constituents *viz*., Flavonoids (Hesperidin), Tannins (Ellagic acid), Glycosides (Glycyrrhizic acid) and Aromatic oils (Geraniol).

Acute Dermal Toxicity: The test substances were applied to the skin region of all the animals uniformly over an area that is approximately10 % of the total body surface area. Test substances held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-h exposure period. The test site was further covered with a suitable gauze dressing to retain the test substance. The maximum dose was 1000 mg/kg for a period of 24 h will be allowed between the testing of each animal. All animals were observed for up to 14 days. 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 behavior pattern

Topical application of Benzoic acid and Cinnamon acid to all groups after that animals produced signs of urticaria which is characterized by redness, itching, swelling. A dose-dependent increase in ear thickness was observed. Topical treatment of phytochemicals reduces redness of guinea pig, ear thickness; it is significant when compared with the treatment of guinea pigs with standard drug.

Gross macroscopic examination revealed a relatively swollen ear in the guinea pig model as compared to that of phytochemicals treated animals. Histopathological examination of guinea pig ear belonging to the disease control revealed prominent epidermal hyperplasia and marked infiltration of inflammatory cells. The guinea pig ear of vehicle control animals exhibits a thin epidermal layer.

Ear thickness was found to be significantly decreased in both test groups when compared to disease control group. Ear thickness of disease induced animals treated with phytochemicals revealed a decreased thickness.

TABLE 1: EFFECT OF PHYTOCHEMICALS ON EAR THICKNESS IN THE BENZOIC ACID (10%) INDUCED NICU IN GUINEA PIGS

Treatment Group	Ear thickness (mm)			
	Hesperdin	Ellagic acid	Glycyrrhizic acid	Geraniol
Vehicle control	0.10±0.0067	0.10±0.0067	0.10±0.0067	0.10±0.0067
Disease control	0.42±0.013	0.42±0.013	0.42±0.013	0.42±0.013
CPM (0.1%)	0.19±0.016	0.19±0.016	0.19±0.016	0.19±0.016
1%w/w	0.27±0.01****	0.318±0.015****	0.318±0.015****	0.266±0.015****
2%w/w	0.19±0.008***	0.214±0.009****	0.214±0.009****	0.154±0.014****

Note: Values are expressed in Mean ± SEM (n=5); Data are analyzed by two -way ANOVA (Tukey's multiple comparisons test) Ear thickness ****P<0.001 *vs.* disease control, time for reducing edema ****P < 0.0001 *vs.* DC (Disease control)

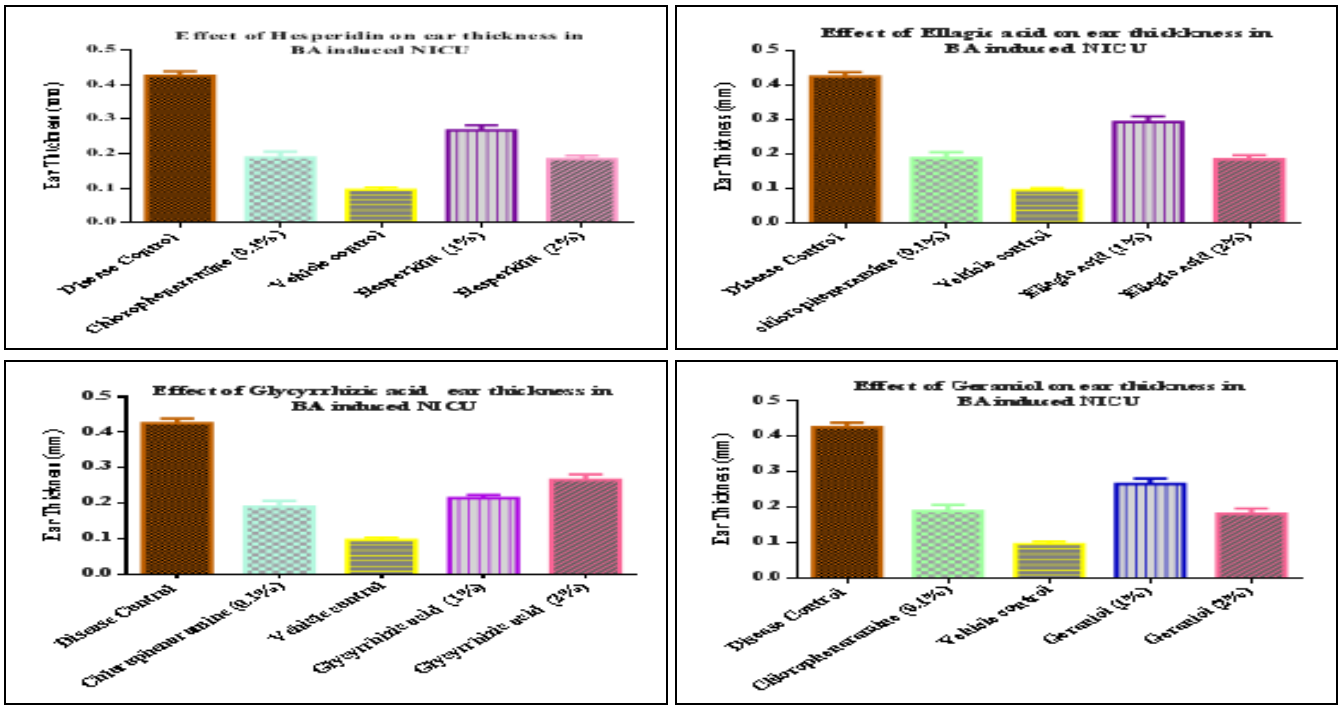

FIG. 1: EFFECT OF PHYTOCHEMICALS ON-EAR THICKNESS IN BA INDUCED NICU IN GUINEA PIGS

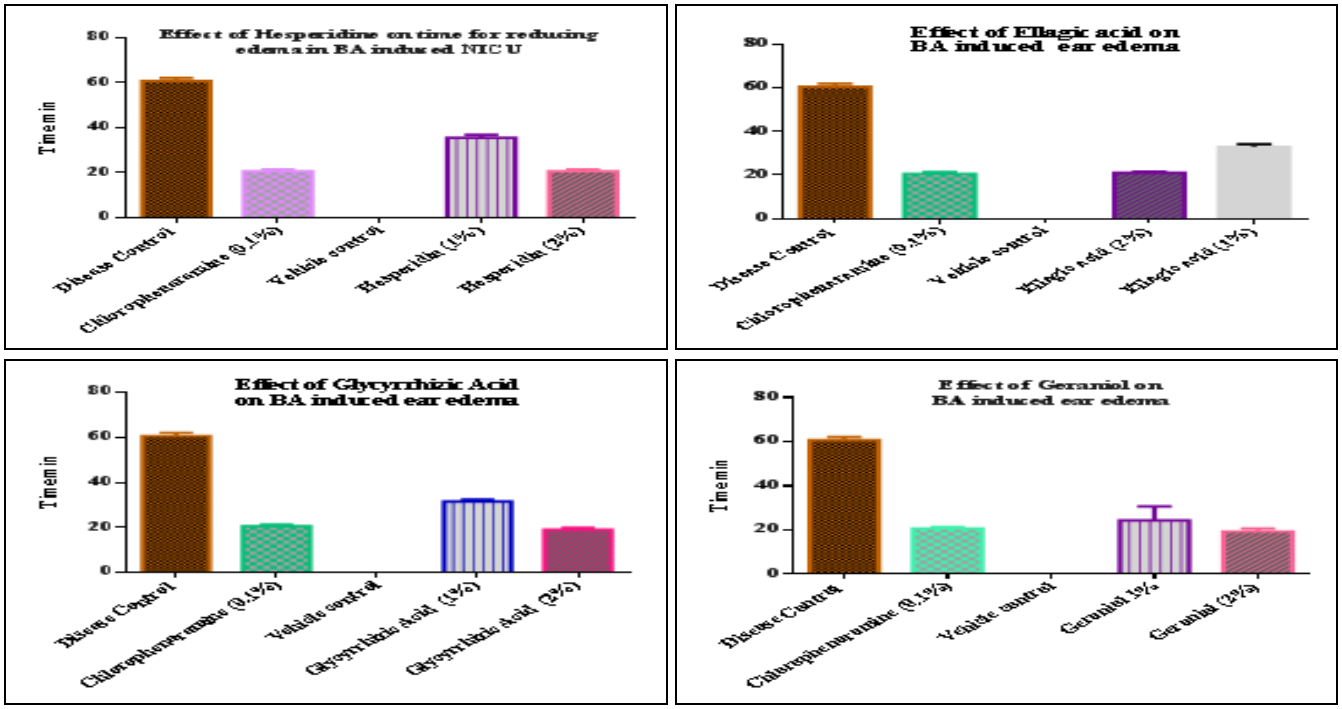

FIG. 2: EFFECT OF PHYTOCHEMICALS ON ERTHYMA IN BA INDUCED NICU ANIMALS

TABLE 2: EFFECT OF PHYTOCHEMICALS ON BENZOIC ACID (10%) INDUCED EDEMA IN GUENIA PIG EARS

Treatment Group	Time (min)			
	Hesperdin	Ellagic acid	Glycyrrhizic acid	Geraniol
Vehicle control	0.0	0.0	0.0	0.0
Disease control	0.40±1.35	0.40±1.35	0.40±1.35	0.40±1.35
CPM (0.1%)	0.20±0.748	0.20±0.748	0.20±0.748	0.20±0.748
1%w/w	0.35±1.2****	0.31±0.87****	0.31±0.87****	0.30±1.93****
2%w/w	0.20±0.92****	0.19±0.948****	0.19±0.948****	0.19±1.28****

Note: Values are expressed in Mean ± SEM (n=5); Data are analyzed by two -way ANOVA (Tukey's multiple comparisons test) Ear thickness ****P<0.001 *vs.* disease control, time for reducing edema ****P < 0.0001 *vs.* DC (Disease control)

TABLE 3: EFFECT OF PHYTOCHEMICALS ON EAR THICKNESS IN GUINEA PIG OF ON CINNAMIC ACID (10%) INDUCED NICU

Treatment Group	Time (min)			
	Hesperdin	Ellagic acid	Glycyrrhizic acid	Geraniol
Vehicle control	0.10±0.0067	0.10±0.0067	0.10±0.0067	0.10±0.0067
Disease control	0.4±0.02	0.4±0.02	0.4±0.02	0.4±0.02
CPM (0.1%)	0.19±0.007	0.19±0.007	0.19±0.007	0.19±0.007
1%w/w	0.292±0.02****	0.31±0.01****	0.312±0.006****	0.32±0.01****
2%w/w	0.198±0.09****	0.206±0.013****	0.238±0.009****	0.20±0.007****

Note: Values are expressed in Mean ± SEM (n=5); Data are analyzed by two -way ANOVA (Tukey's multiple comparisons test) Ear thickness ****P<0.001 *vs.* disease control, Time for reducing edema ****P < 0.0001 *vs.* DC (Disease control)

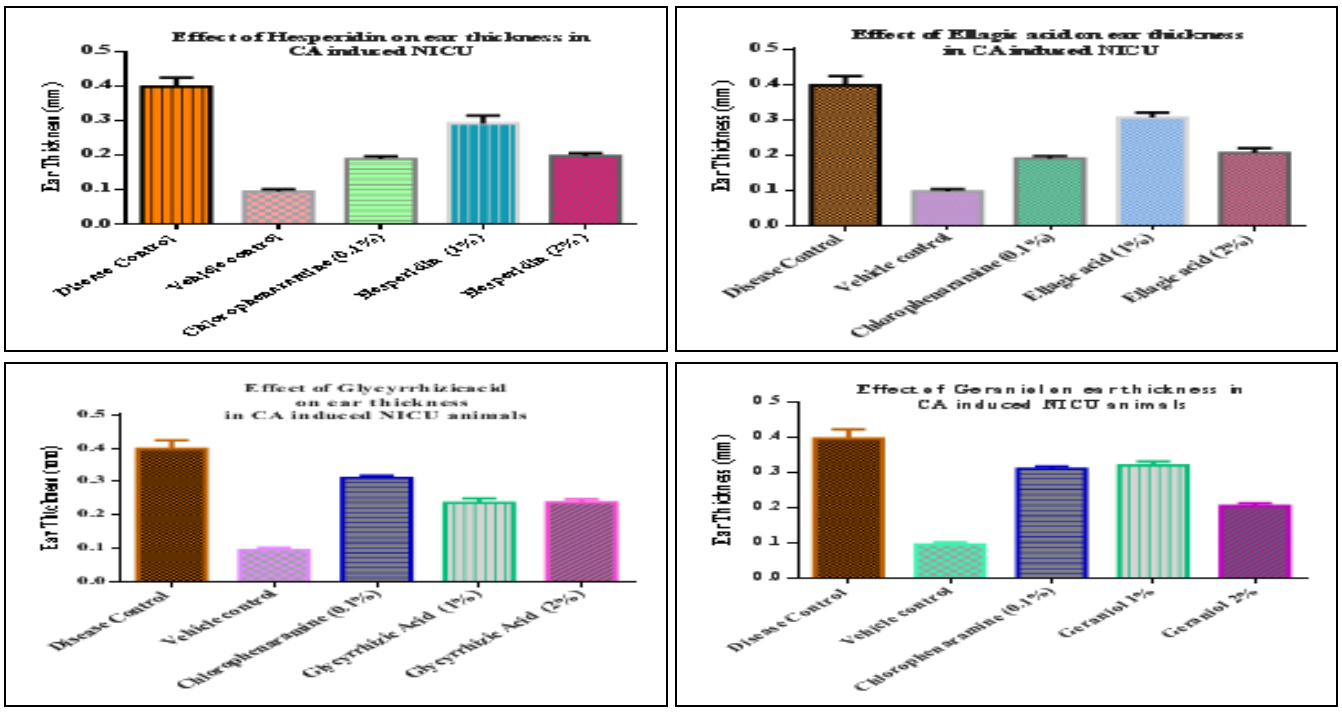

FIG. 3: EFFECT OF PHYTOCHEMICALS ON EAR THICKNESS IN CA INDUCED NICU ANIMALS

TABLE 4: EFFECT OF PHYTOCHEMICALS ON EAR THICKNESS IN GUINEA PIG OF ON CINNAMIC ACID (10%) INDUCED NICU

Treatment Group	Time (min)			
	Hesperdin	Ellagic acid	Glycyrrhizic acid	Geraniol
Vehicle control	0	0	0	0
Disease control	0.436±1.53	0.436±1.53	0.436±1.53	0.436±1.53
CPM (0.1%)	0.186±0.927	0.186±0.927	0.186±0.927	0.186±0.927
1%w/w	0.35±1.64****	0.32±1.2****	0.30±1.2****	0.30±1.93****
2%w/w	0.20±0.73****	0.21±1.28****	0.19±0.707****	0.19±0.81****

Note: Values are expressed in Mean ± SEM (n=5); Data are analyzed by two -way ANOVA (Tukey's multiple comparisons test) Ear thickness ****P<0.001 *vs.* disease control, time for reducing edema ****P < 0.0001 *vs.* DC (Disease control).

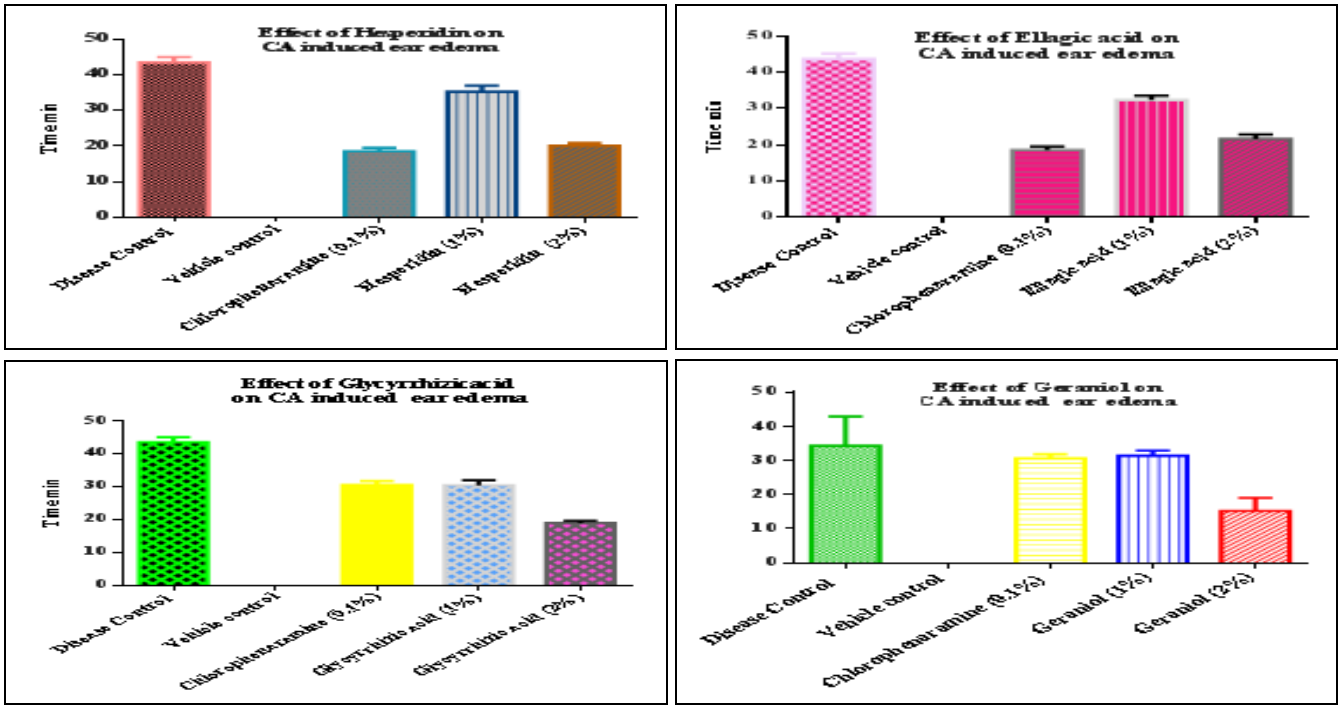

FIG. 4: EFFECT OF PHYTOCHEMICALS IN TIME FOR REDUCING ERTHYMA IN CA INDUCED NICU ANIMAL

DISCUSSION: The effect of phytochemicals was evaluated in BA, CA -induced NICU by topical administration. The disease control group animals (Group-I) showed redness, itching, swelling after application of BA, CA ointment to ears of animals. Ear thickness was found to be significantly increased (two to three folds) in the disease model as compared to vehicle control. Chloropheniramine used as the standard drug at the concentration of 0.1% potently decreased chemical-induced NICU with a suppressive rate 60%. Test compounds 1% and 2% decrease the time to reduce edema induced by BA& CA on animal ears. Phytochemicals potently reduced the urticaria within 19 min (**Table 1** and **3** and **Fig. 1** and **3**). The suppressive rate of edema by phytochemicals at 1% concentration was 12.5%, 23.5%, 23.5 & 25% and at 2% concentration was 50%, 53.5%, 53.5% and 53.5% on respectively as compared to the disease control animal in BA inducing NICU.

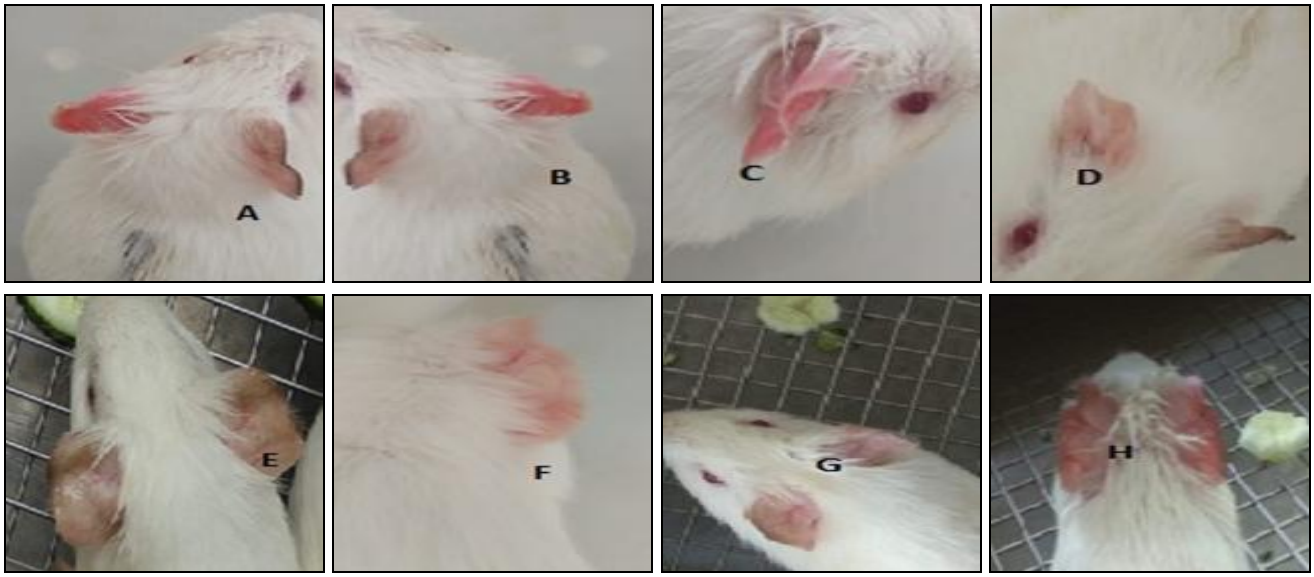

FIG. 5: EFFECT OF PHYTOCHEMICALS ON THE NICU INDUCED BY BENZOIC ACID & CINNAMIC ACID (10%). (A). ANIMAL TREATED WITH BA. (B). ANIMAL TREATED WITH CA. (C). DISEASE CONTROL ANIMAL EAR AT THE END OF WORK. (D). ANIMAL TREATED WITH STANDARD (E). ANIMAL TREATED WITH 2% GERANIOL. (F). ANIMAL TREATED WITH 2% GLYCYRRHIZIC ACID (G). ANIMAL TREATED WITH 2% ELLAGIC ACID (H) ANIMAL TREATED WITH 2% HESPERIDIN

The suppressive rate of ear thickness by phyto-chemicals at 1% concentration was 27.5%, 22.5%, 22% and 20% and at 2% concentration was 42.5%, 50%, 43.5%, and 50% on respectively as compared to the disease control animal in BA inducing NICU.

The suppressive rate of edema by phytochemicals at 1% concentration was 28.7%, 26.7%, 25%, and 25% and at 2% concentration was 54.2%, 51.9%, 56.5% and 56.5% on respectively as compared to the disease control animal in CA inducing NICU. The suppressive rate of ear thickness by phytochemicals at 1% concentration was 26.5%, 22.5%, 22 and 20% and at 2% concentration was 50.5%, 50%, 41.5%, and 50% on respectively as compared to the disease control animal in CA inducing NICU. Animals treated with STD at the concentration of 0.1% decreased ear thickness by 56.5%. Topical treatment of phytochemicals at 1% & 2% w/w ointment preparations significantly (****$p<0.0001$) reduced BA & CA induced NICU on animal skin by reduced redness of the skin and ear thickness.

CONCLUSION: Urticaria was induced in the ear of a guinea pig with the help of inducing agents like benzoic acid and cinnamic acid. The urticarial induced was accompanied by sustained ear, redness, swelling, and itching. All phytochemicals reduced the redness, ear thickness at 1% and 2% w/w rapidly and effectively, which is similar to that of standard drug. When compare to 1% w/w phytochemicals at 2% drastically reduced erythma and urticaria on skin of animal. Based on results, it was concluding that selected phytochemicals at 2% had good activity against NICU. These compounds are quite useful for the treatment of chemical inducing urticaria.

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