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PHYTOCHEMICAL SCREENING AND ANTI-PSORIATIC EVALUATION OF CITRUS SINENSIS PEEL EXTRACTS ON MOUSE TAIL MODEL

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ABSTRACT

Objective: To assess anti-psoriatic activity of the ethanol and aqueous extract from the peels of *citrus sinensis* on mouse tail model. **Methods:** Mouse tail test using Perry scientific tail model was used for the evaluation of anti-psoriatic activity. Aqueous extract and ethanol extract of (100mg/kg) were tested in Swiss albino mice. Parameters studied in the mouse tail test were changes in epidermal thickness and percentage orthokeratotic values. **Results:** The extracts from the peels of *citrus sinensis* produced significant orthokeratosis (P<0.01) in the mouse tail test. In epidermal thickness, a significant reduction with respect to control was observed in groups treated with flucinolone acetonide and ethanolic extract. **Conclusion:** From the above data, the extracts of *citrus sinensis* showed significant orthokeratosis on mouse tail test. To our knowledge, this is the first report on the anti-psoriatic effect of peels of *citrus sinensis.*

KEYWORDS: Citrus sinensis, flucinolone acetonide, orthokeratosis.

INTRODUCTION

It is generally recognized that by our ancestors that a wide range of medicinal plants have healing powers. Until the 20th century every village and rural community had a wealth of herbal folklore. They have tried and tested local plants for a range of common health problems. They were using herbal plants as teas and lotions or even mixed with lard and rubbed in as an ointment. People were aware of good and bad effect of herbal plants. For example, eating of a particular root, leaf or berry by watching animal behaviors after they

have eaten or rubbed against certain plant. Those observations have also added to medicinal values of the forest plants.

The pharmaceutical industries have made massive investment in pharmacological clinical and chemical researches all over the world in past five decades. Effort has been made to discover still more potent plant drugs. In fact a few new drug plants have successfully been passed the tests of commercial screening.

Psoriasis an immune-mediated, inflammatory skin disorder characterized by red, thickened plaques with overlying silvery-white scaly patches mainly distributed into extensor surfaces and may also involve palms and scalp.[1] Psoriasis can be initiated by certain environmental triggers. A predisposition for psoriasis is inherited in genes.[2] Psoriasis is not contagious but can be inherited. Research indicates that the disease may result from a disorder in the immune system. Psoriasis gets better and worse spontaneously and can have periodic remissions (clear skin). Psoriasis is controllable with medication. Psoriasis is currently not curable.[3] There are many promising therapies, including newer biologic drugs. Future research for psoriasis is promising. Psoriasis is a common and chronic incurable but treatable skin disorder.[4]

Plant Monograph

Orange tree (aka Sweet orange) is a species of flowering plants with the scientific name *Citrus sinensis*. It is one of many different types of citrus plants and one of the main crops grown for income in Florida. The orange fruit is actually a reproductive ovary of the tree. The orange, although grown in many places in the world, actually originated from Asia. Of the sixteen types of the genus *Citrus*, the Citrus sinensis is the most important of the citrus fruits.[5,6]

Taxonomical Classification

kingdom : plantae

sub kingdom : angiosperms
phylum : eudicots
division : magnoliophyta
class : magnoliopsida
order : sapindales
family : rutacea
genus : citrus
species : citrus sinensis

"Phytochemicals: Secret Nutrients to Health"

For the most part, we are familiar with the basic nutrients in our foods; proteins, carbohydrates, fats, vitamins, minerals and water. However, plant foods, especially colorful fruits and vegetables, also contain "secret nutrients" that play an important role in health and disease prevention.[7]

It is under consideration and a long list of health organizations are taking a closer look at photochemical. It's listed as flavonoids, terpenes, indoles, phenolic acids, isothiocynates etc.[5] But these tongue twisters are not artificial additives, and they are naturally occurring chemicals found in foods like fruits, vegetables and grains, which our body apparently uses as part of their disease fighting arsenals.[8,9]

In the present study, an attempt was made to evaluate the anti-psoriatic activity of sweet oranges peel extract (both ethanolic and aqueous) from the fruits of citrus sinensis, on rat tail model. Which is listed in indigenous medicine as having high therapeutic value and is even now being used in various diseases.[10] It is also called as "The fountain of youth".

MATERIAL AND METHODS

Collection of sample: Fresh sweet orange were collected from the local streets of Guntur in the month of December 2016. The handpicked Sweet orange were washed well using tap water and twice using distilled water. Then the peel and pulp of Sweet orange were separated by cutting them into small pieces and it was dried in shade for a period of 20-25 days, at an ambient temperature of 30˚C. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form.[11,12]

Preparation of the extracts

Aqueous extract

The method adopted for extraction Briefly, 15g of the powdered plant were soaked separately in 200 ml of distilled water at room temperature for 24 hour under shaking condition. The extract was then filtered using Whatman filter paper No.1 then concentrated to dryness by using the water bath at 70ºc. Yield of the extract is weighed on the weighing balance. Each extract were transferred to glass vials and kept at 4ºC before use.[13,14]

Soxhlet Extraction

Orange fruits were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 40°C for 48 h and ground to a fine powder and passed through a 24-mesh sieve according to the method described by Van-Acker *et al.* 100g powdered sample was extracted with either 250ml ethanol or methanol at room temperature by Soxhelt extraction method for 6 h. The mixture filtered through a Whatman No. 2 filter paper for removal of peel particles. The residue was re-extracted twice under the same condition to ensure complete extraction. The extracts were filtered and evaporated to dryness under reduced pressure at 60°C by a rotary evaporator. The extracts were placed in dark bottles and stored in refrigerator at 4°C until use.[16,22]

Experimental animals

Healthy male adult albino mice (25–30 g) obtained from the Animal, India were used for the study. Animals were housed in polypropylene cages and were left 7 days for acclimatization to animal room maintained under controlled condition [a 12 h light-dark cycle at (22±2°C)] on standard pellet diet and water. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with due approval from the Institutional Animal Ethics Committee. Registration no 1048/a/07 CPCSEA Animals were used for acute toxicity study and mouse tail test for psoriasis.

Extracts tested

The ethanolic extract and aqueous extract from the peels of C.*sinensis* were screened for anti-psoriatic activity. The ethanolic extract (100 mg/kg) and Aqueous extract (100 mg/kg) were formulated in the form of a cream, using liquid paraffin (10 mL) and bees wax (3 g) and applied topically. Flucinolone acetonide 0.025% w/v cream (Flucort cream I.P.) Glen-mark Pharmaceuticals was used as a standard, and saline water is used as control.[15,24]

Acute toxicity studies

Acute toxicity study-up and down procedure was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD). Mice (4/group) were divided into five groups. The first 2 groups received topical doses of 100mg/kg, of ethanolic extract and aqueous extract the 3rd group received topical doses of standard flucinolone. The 4th group received saline water tropically. Mortality was assessed 24 h after administration. The animals were also observed for toxic symptoms and mortality was determined 24 h after treatment.[18]

In vivo anti-psoriatic activity

The mouse-tail model is based on the induction of orthokeratosis in those parts of the adult mouse-tail, which have a normally parakeratotic differentiation.[23]

Perry scientific mouse tail model

This is accepted as a screening method for measuring anti-psoriatic activity of drugs. The basis of this method is that topical treatment of a mouse-tail with anti-psoriatic drugs enhances orthokeratotic cell differentiation in the epidermal scales. This characteristic was utilized for direct measurement of drug efficacy in an animal model. Drugs were applied topically, once daily, 5 times in a week, for 2 weeks. Two hours after the last treatment, the animals were sacrificed; longitudinal sections of the tail skin were made and prepared for histological examination (hematoxylin- eosin staining). As an indicator of orthokeratosis, the number of scale regions with a continuous granular layer was counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.[17,19]

Procedure

Healthy male adult albino mice (25–30 g) used. Screening of ethanolic extract and aqueous extract (in the form of cream) was carried out with reference to the standard, flucinolone 5%. Extract and fraction (in the form of cream) were applied topically, once daily, 5 times a week, for 2 weeks. tests were applied topically, once daily, 5 times a week, for 2 weeks. Two hours after the last treatment, animals were sacrificed, longitudinal sections of the tail skin were made and prepared for histological examination (hematoxylin-eosin staining). As an indicator of orthokeratosis, the number of scale regions with a continous granular layer was counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.[20,21] Ten sequential scales

were examined for the presence of a granular layer induced in the previously parakeratotic skin areas.[20] The induction of orthokeratosis in those parts of the adult mouse tail, which have a normally parakeratotic differentiation, was quantified measuring the length of the granular layer (A) and the length of the scale (B). The proportion (A/B)×100 represents the % orthokeratosis per scale, and the drug activity (DA) was calculated as follows:

- DA = (mean OK of treated group − mean OK of control group × 100) / (100 − mean OK of control group)
- Where OK means orthokeratosis, The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit.

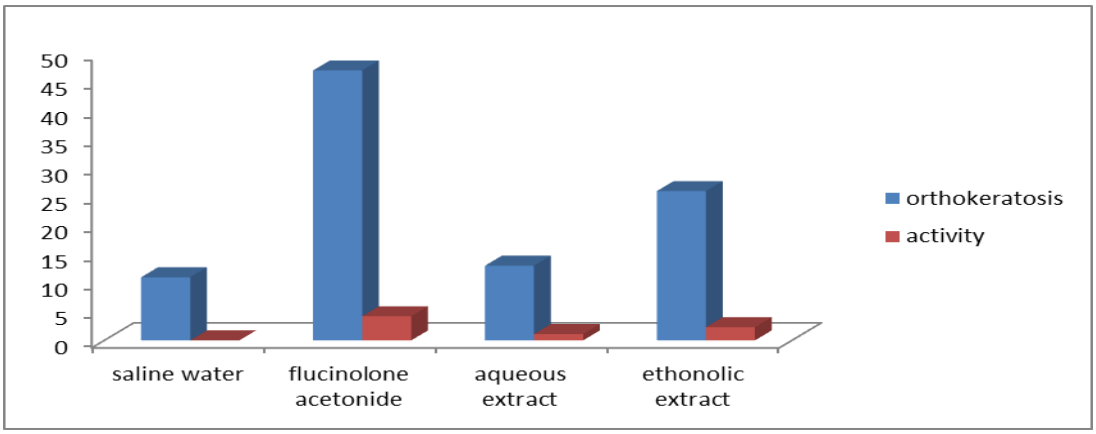
Measurement of epidermal thickness

It was obtained by measuring the distance between the dermo epidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales and the mean of different animals was calculated.[25] The result of extracts both ethanol and aqueous, against psoriasis was recorded.

RESULTS AND DISCUSSION

Table no 1: Effect of aqueous and ethanolic extract of citrus sinensis peels on the degree of orthokeratosis, and the 'drug activity' in the mouse tail test (mean±SEM) (n=4).

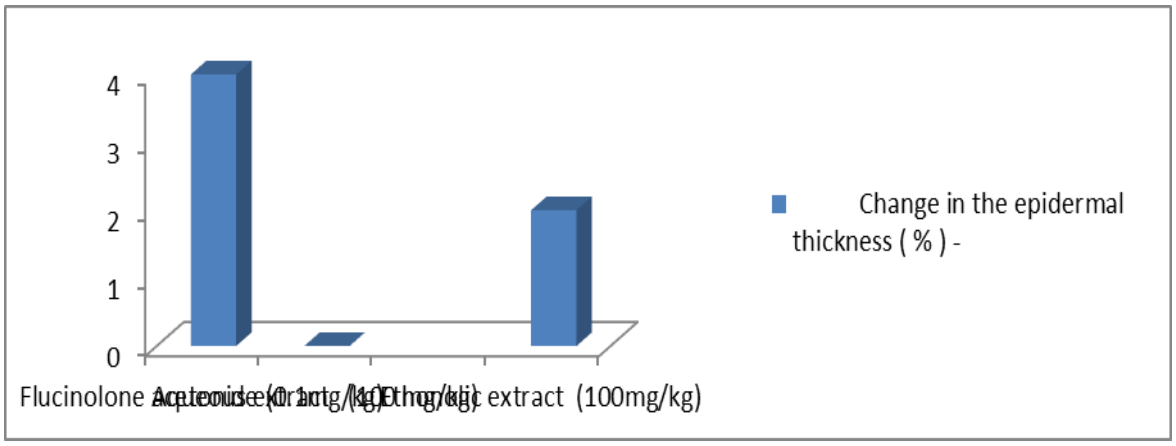
S.No	Treat ments used	Orthokeratosis(%)	Activity(%)
1	Saline water	11±3.20	-
2	Flucinolone acetonide (0.1mg/kg)	47.06±1.09	4.2
3	Aqueous extract (100mg/kg)	13.06±2.90	1.1
4	Ethonolic extract (100mg/kg)	26.86±3.10	2.3



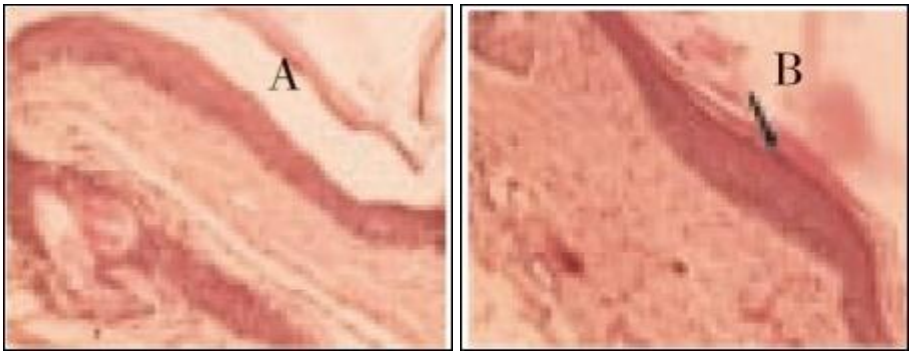
Graph No 1: Graphical representation of Activity of various extracts which are defined by increase in orthokeratosis.

Table No 2: Effect of aqueous and ethanolic extract of citrus sinensis peels on the change of epidermal thickness in the mouse tail test (n=4).

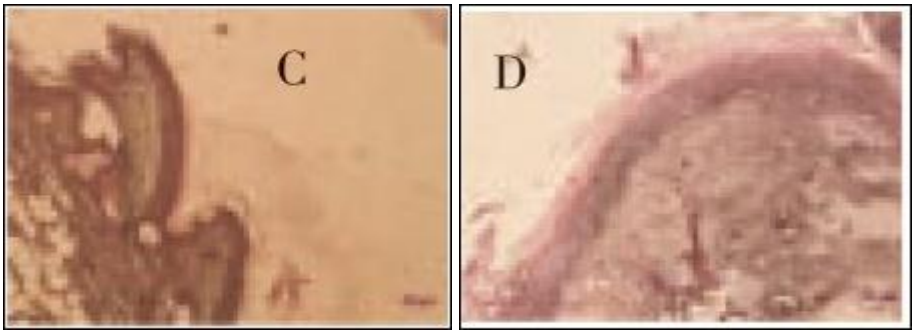
S.no	Treatments	Change in the epidermal thickness (%)
1	Saline water	-
2	Flucinolone acetonide (0.1mg/kg)	4
3	Aqueous extract (100 mg/kg)	0
4	Ethonolic extract (100mg/kg)	2



Graph No: 2 ***Graphical representation of change in epidermal thickness.***



A. Saline water treated. B. Flucinolone acetonide treated.



C. Aqueous extract treated. D. Ethanolic extract treated.

T.S Longitudinal histological sections through the skin of mouse tails treated Topically for two weeks HE staining (original magnification 40×).

Evaluation of Anti-Psoriatic Activity

The Aqueous and ethanolic extracts of citrus sinensis peels were screened for their possible anti-psoriatic activity using Perry's scientific mouse tail model. Extracts were applied topically in the form of a cream. Drug activity is defined by the increase in percentage of orthokeratotic regions. These are the regions in a cell having no nucleus and involved in protection from invaders like micro-organisms, UV rays, weak acids and bases. Aqueous extract (100 mg/kg) increased the orthokeratotic regions by 13.06%, respectively, whereas 26.86% by ethanolic extract (100mg/kg) in comparison to normal, The standard drug flucinolone showed the increase by 47.06%.

DISCUSSION

The therapeutic potential of flavonoids and the necessity for scientific validation in popular medicine have prompted increased interest in the field. Citrus sinensis, a hesperidium belonging to the Rutaceae family is a food component with proven beneficial impact on health.[21] Hesperidine a flavan-on glycoside has many pharmacological activites such as. Aqueous and Ethanolic extracts are obtained from the citrus sinensis peels and were evaluated for antipsoriatic efficacy using mouse tail test. In the mouse tail test. Ethanolic extract (100 mg/kg) produced significant orthokeratosis when compared to control. The Ethanolic extract also showed slight change in epidermal thickness compared to control. While Aqueous extract did not produce any significant change in epidermal thickness. Granular layer of the epidermis is greatly reduced or absent in psoriatic lesions.[22] Parakeratotic condition is seen in the adult mouse tail which is one of the hallmarks of psoriasis.[23] Induction of orthokeratosis in the adult mouse tail is the basis behind the mouse tail test.

CONCLUSION

The aqueous as well as the ethanoic extracts of the peel revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, terpenoids, steroids, and amino acids where as the saponins are present only in the ethanolic extracts. From the pharmacological studies and the data of results obtained, both the test formulations (ethanolic extract and aqueous extract) of citrus sinensis alleviated the signs of potency to treat psoriasis by significant increase in the orthokeratosis. The results showed that the antipsoriatic activity of the *Citrus sinensis* peel extracts was due to the presence of the rich amount of bioactive phytoconstituents i.e flavonoids and polyphenols in the extracts. These findings suggest the

potential for the use of *Citrus sinensis peel extracts* in the treatment of psoriasis, confirming their traditional use in skin disorders. Further studies are required to gain more insight to the isolated phytoconstituents. To our knowledge, this is the first report on the anti-psoriatic effect of the Citrus sinensis peel which needs further investigation to prove its anti-psoriatic activity.

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