Biological Evaluation of *Nigella Sativa* and *Cydonia Oblonga* Seeds Extract for Anti-Psoriatic Activity against UV-Induced psoriasis in rodents

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Abstract

To evaluate the synergetic potential of *Nigella sativa* and *Cydonia Oblonga* seeds extract for anti-psoriatic activity in UV-induced psoriatic rats. Five groups of animals each contain 5, vehicle control, disease control, Standard group, Test I (5% ointment), Test II (10% ointment). Observed epidermal thickness, Total leucocyte count, IL-6 of animals later completion of the treatment period. Animals received test compound at 5% was produce the mild effect on the psoriatic lesions by reducing the inflammation and itching as compared to the disease control. In case of test compound at 10% was produce the significant reduction in the epidermal thickness and PSI, TLC and IL-6 while compare with the disease control. Over all study conclude that seed extract of *Nigella sativa* and *Cydonia Oblonga* having synergistic effect on UV-induced Psoriasis in Laboratory animals, these could be that best combination might be useful for clinical in psoriatic people for better therapeutic outcome.

Keywords: *Nigella sativa, Cydonia oblonga*, Anti-psoriatic activity, Flavonoids,

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Introduction

The skin, the biggest organ in the body, serves as an animal's first line of defence against diseases and germs. The outer epidermis and the inner dermis are the two main layers that make it up. A layer of keratinocytes that are closely knit in the outer epidermis act as a barrier against pathogens and different environmental stressors. Additionally, it is made up of resident immune cells and melanocytes, which both perform a similar function by maintaining the barrier that keep hazardous substances out. Melanocytes are particular melanin-producing cells that give skin its colour. According to recent studies, our epidermis layer is fully enclosed by the commensal microorganisms necessary to maintain the skin homeostasis (1)(2). Psoriasis is a skin disorder that affects the scalp and palms in addition to extensor areas and is characterised by thickened plaques, redness, and overlaying silvery-white scaly patches. Certain environmental stimuli, chemical and gene abnormality may exuberate the condition (3)(4).

Psoriasis susceptibility through genetically is much higher than other etiological risk. Although it cannot be transmitted from one person to person by personal contact, it could be inherited. Previous studies have demonstrated that the immune system's disarray may be the cause of the ailment. If psoriasis sufferers experience regular remissions, the condition may worsen and become suboptimal (5). Women are more likely than males to develop psoriasis early in life. It typically occurs in adults betweenthe ages of 16 and 22 and is referred to as a first peak. People beyond the age of 57 experience the second peak. It might have an equivalent impact on all races. Numerous promising treatments existed, including more recent biologic medications (6)(7). Because it is a common and persistent skin condition that is incurable yet treatable, future research in this area seems promise. Having one parent with psoriasis increases your risk of getting the disease, and having two parents with psoriasis increases your risk even more (8). Because stress can impact your immune system, high stress levels may increase your risk of psoriasis. Smoking

tobacco not only increases the risk of psoriasis but also may increase the severity of the disease. It also plays a major role in the initial development of the disease (9).

Abnormal keratinocyte differentiation, and excessive Th-1 and Th-17 inflammation ensue. Initial signs of psoriatic lesions include intra-epidermal perforation of activated polymorphonuclear leukocytes, which causes unchecked production of reactive oxygen species (ROS), it may induce the peroxidative damage to skin membranes and progresses to worsening of lesions (10). Additionally, ROS can cause phospholipase A2 (PLA2) to be activated, which will encourage the production of inflammatory mediators from arachidonic acid (AA). By widening dermal capillaries, boosting leukocyte infiltration, and promoting keratinocyte cell development, prostaglandin E2 (PGE2) produced by the cyclooxygenase (COX) pathway may provoke the psoriasis (11)(12).

Currently majority of physicians are prescribing the Salicylic acid ointment to people with psoriasis lesions, because it smooths the skin by accelerating the remove the psoriatic scales. Steroid creams are the predominant method of treating psoriasis; they reduce inflammation, relieve itching, and reduce the generation of cells that are overproduced in psoriasis people (13). The present study is to evaluate the anti-psoriatic activity of combination of *Nigella sativa* and *Cydonia oblonga* seeds extract in Wistar rats.

Materials and methods

Animals: Wistar rats of both gender with 150-200gm body weight

Chemicals: Salicylic acid**,** plant extracts

Methodology

All the experiment was carried out on Wistar rats which were divided into 5 groups and each group contains (n = 5). The animals were kept in separate cages for acclimatization at a temperature of 25±2℃ and relative humidity of 52-55% with 12h light/dark cycle 1week before commencement of the experiment. During this period animal received standard pellet diet ad libitum and drinking water throughout the study period. The protocol of experimental study was approved by the Institutional Animal Ethics Committee of Chalapathi institute of Pharmaceutical Sciences, with IAEC approval no: 05/IAEC/CLPT/2021-22, on (Dt: 01/02/2022).

These

groups

are

Induction of Psoriasis by UV Rays: To induce psoriasis for laboratory animals, animals were exposed daily with contact time of 10 sec under the UV-rays for 14 days. After completion of period, all animals are found with lesion, scaly patches, erythema on the dorsal surface of body. It is similar to the psoriatic lesion found in the human beings. Later the animals were treated with respective standard, test compound at two concentrations. (14)

Preparation of simple ointment/ ointment with test compounds

The formulation developed was an ointment of two different concentrations i.e. 5% and 10%. Primarily simple ointment was prepared by the fusion method. The preparation of simple ointment 100g base by using the hard paraffin(5g), Cetostearyl alcohol(5g), white soft paraffin(85g) and wool fat(5g) were added in descending order of their melting points respectively after removing from melting container, the ointment was formed. From this the ointment was separated and extracts were added as per the concentrations required. Finally, ointments containing 5% and 10% concentration were made using the extracts and simple ointment base as vehicle. (15)

Acute dermal toxicity

Healthy young adult animals of commonly used laboratory strains were employed. The selected animals were 8–12 weeks old and the weights 150-200gm.The animals were acclimatized to the laboratory conditions for 7 days before the start of the study. Animals are randomly selected for the study and marked to provide individual identification. Approximately 24 h before the study, fur was removed from the dorsal area of the trunk of the animals by using epilator cream. The test substance was applied uniformly over an area which is approximately 10% of the total body surface area. Test substances were held in contact with the skin with the help of porous gauze dressing and non-irritating tape throughout a 24 h exposure period. Animals were observed for signs of toxicity from 30 min to periodically during the first 24 h, with special attention for the first 4 h. All animals were free from signs of irritation, itching, redness and allergic symptoms on the applied area. This confirm that sample is free from acute dermal toxicity.

Determination of anti-psoriatic activity

All the animals were divided into 5 groups, each group containing 5 animals.

treated as follows:

Research design

Rats of both gendered were collected and divided into 5 groups, each group with 5 animals.

a) These groups are treated as group-1(control animal), group-2 (standard drug treated animals), group-3 (negative control), group-4 (5% test drug), group-5(10% test drug).

b) Salicylic acid ointment was applied to the dorsal surface of the animals in standard group.

c) Remaining group animals were treated with respective test compounds.

The animals were found with psoriatic lesions, sliver scaly patches after completion of UV exposure. Later each group animals were treated with respective drugs for 14 days. The vehicle control group with ointment base, the standard with 2% salicylic acid ointment and the remaining groups with 5 & 10% Test-I, Test-II, respective groups. Later the rats were sacrificed for the tail and skin for the histopathology studies. For therapy, dorsal skin was treated with phytochemicals to all of group of animals excluding vehicle control. After completing treatments with phytochemicals last day, all of the rats were sacrificed and skin tissues at the lesion sites were isolated and sent to histopathological studies. By observing the sign on skin, all animals were assessed for Psoriasis severity daily up to the 7th-day treatment period by following the Psoriasis Area Severity Index (PASI). Scores were given as follows: 0 there is no psoriasis, 1 mild, 2 moderate, 3 severe and 4 very severe (15).

Results

1.Phytochemical analysis of seed extracts

Table No: 1. Phytochemical constituents present in seed extract of *Nigella sativa & Cydonia oblonga*

S.no	Chemical tests	*Cydonia oblonga*	*Nigella sativa*
1	Alkaloids	_	+
2	Flavanoids	_	+
3	Terpenoids	_	+
4	Tannins	+	+
5	Glycosides	+	+
6	Phenolic compounds	+	+
7	Coumarins	_	+
8	Carbohydrates	+	+

The phytochemical studies confirms that flavonoids in the ethanolic extract of *Nigella sativa* and *Cydonia oblonga* found the carbohydrates present in the mucilage of *Cydonia oblonga* where the main focus on the research actually exists. Along with the flavonoids and carbohydrates terpenoids, tannins, phenolic compounds are also identified based on the respective chemical tests. (16)

2. Acute Toxicity studies

A) Primary Irritation Testing: The formulated ointment doesn't cause any irritation when applied to the skin.

B) Acute dermal toxicity: Acute dermal toxicity was carried out according to OECD guideline 402 (2). After completion of 14 days of treatment, it was found that none of the animal doesn't shows any signs of gross lesions, body weight changes and rashes on dorsal skin. The developed formulation was found to be free from acute dermal toxicity (17).

3. Pharmacological screening

Anti-Psoriatic Activity (UV-Induced Psoriasis in Wistar rat)

Table.No.2: Effect of NSSE and COSE on the epidermal thickness, on UV-induced psoriatic rat. (18)(19)

S. No	Treatment group	Epidermal thickness (mm)
1.	Vehicle Control	12.96 ± 0.196****
2.	Disease control	35.42 ± 0.540****
3.	Standard	22.56 ± 0.696****
4.	Test-1 (5%)	31.86 ± 0.497****
5.	Test-2 (10%)	29.44 ± 0.461****

Note: Values are Mean ± SEM; epidermal thickness was significantly decreased than thecontrol group, Data are analysed by *one -way ANOVA.* n=5, (**** p< 0.0001)

Table.No.3: Effect of NSSE and COSE on the total leucocyte count in, UV-induced psoriatic animals

S. No	Groups	TLC(*100/mm^3)

1 2 3 4 5	Control Standard Disease control Test -1 Test-2	2.588±0.031 2.784±0.025 3.842±0.041 3.198±0.057 3.058±0.046

P value summary is significant (**** p< 0.0001)

Table.No.4: Effect of NSSE and COSE on the PSI, in UV-induced psoriatic animals

Groups	PSI-1 (I-Week)	PSI-2 (II-week)	PSI-3 (III-week)
Control Standard Disease control Test -1 Test-2	0 3.4±0.10 2.44±0.12 3.38±0.07 3.3±0.04	0 2.6±0.11 3.32±0.05 2.98±0.09 2.72±0.11	0 1.32±0.05 3.72±0.08 2.12±0.08 2.32±0.08

Note: P value summary is significant (**** p< 0.0001)

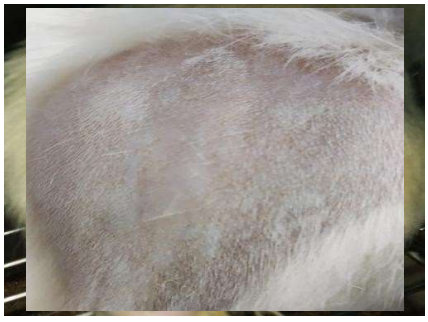
Table.No.5: Effect of NSSE and COSE on the IL, in UV-induced psoriatic animals

S. No	**Groups**	**IL(*pg/ml)**
1	Control	23
2	Standard	65
3	Disease control	154
4	Test -1(5%)	85
5	Test-2(10%)	23

Dorsal view of animal skin: Before treatment

Vehicle control

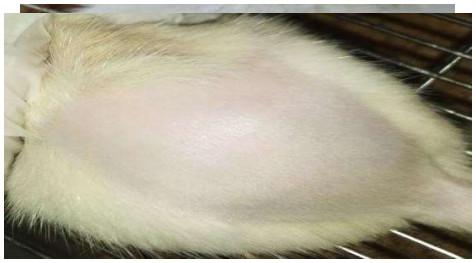
Standard



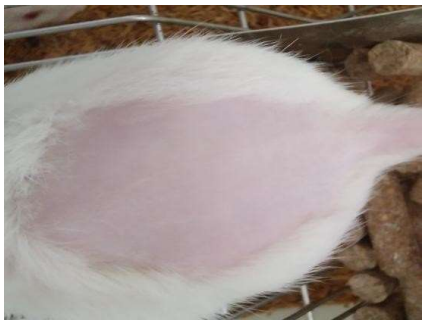
Test 5%

Test 10%

Dorsal view of animal skin: After treatment

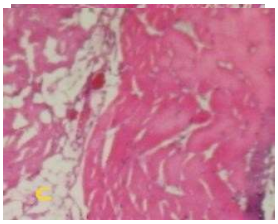


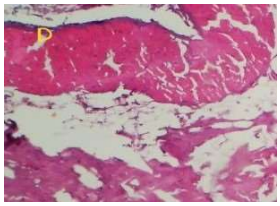
Vehicle control Standard



Test 5 % Test 10 %

Histology of epidermis treated with both standard drug and test compounds





Vehicle control Disease control (UV) Standard(UV ray + SA)

Test 5% Test 10%

Discussion

The seed extract of *Nigella sativa,* and *Cydonia oblonga* are rich in flavonoids, tannins, and phenolic compounds. Various researcher confirmed that both plants having good therapeutic values for treating various disorders in human. (20,21) Similarly, treatment with herbs may having best therapeutic outcome, without any complications which were seen in synthetic formulations. Current study mainly focusses on the evaluation of synergetic effect of NSSE and COSE for treating chronic dermatological disorder psoriasis in laboratory animals.

Animals of all groups were treated with respective compounds with 14 days. During this period animals were observed the skin for recovery of psoriatic lesions gave Psoriatic severity score (PSI) to know the potency and efficacy of test compounds. At the end of the study all animals were scarified by using high dose of CO2, tails are separated collect the tissue for histological examination. (22,23) The mean thicknesses of the epidermis, in disease control- and drug-treated animals were tabulated [Table.No.2]. There was significant decrease in epidermal thickness in NSSE and COSE treated animals while compare with the disease control (****P < 0.0001). The section of disease-control group showed regular elongation of reteridges, capillary loop dilation with minimal grade lesion of diagnostic Munro's microabscess and marked increase in relative epidermal thickness as compared with other groups. In 5%w/w of treated group, there was a minimal grade lesion of elongation of rete

ridges along with capillary loop dilation in the section and absence of Munro's micro abscess (24). In 10 % treated group, there was no lesion of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section of skin of rats (Figure-3). In the standard group, there was absence of Munro's microabscess, capillary loop dilation along with elongation of reteridges in the section showing significant therapeutic effects when compared with test-treated groups. (25,26)

PSI was given to all individual group animals based on their signs and presence of erythema, itching and silvery scales on the skin. Disease Control group gain score of 2.44 at first week, it was increased to 3.72 at the end of the experiment (3rd week), standard group gain 3.4, at the first week, after that application of drug on the topically to the skin, reduction in signs and symptoms of psoriasis slowly and 40 %reduction is seen at 3rd week that 1.32 (Table no-4) (27)(28). Test treated groups all were initially gained the score 3.3 to 3.3 at first week, it was slowly reduced to 2nd week, totally (63%,70%) reduction in PSI is observed at the time of third week of treatment period. It reveals that bio flavonoids, tannins, lipid derived terpenoids have more effective for treatment of chronic disorders of human skin without complications.

Conclusion

The current study offers the pharmacological proof that topical administration of particular phytochemicals can successfully prevent UV induced psoriasis-like skin inflammation in rats. This prevention may be attributable to the inhibition of various cytokines related to Th1 cells, Th17 cells, and keratinocytes, which are known to playcrucial roles in the pathogenesis of human psoriasis. These results support and strengthen the therapeutic justifications for using phytochemicals to treat mild to moderate psoriasis. Further research can be carried out for the phytochemical analysisand the screening

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Conflict of Interest – Nil

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