Antipsoriatic activity and cytotoxicity of ethanolic extract of *Nigella sativa* seeds

Lalitha Priyanka Dwarampudi, Dhanabal Palaniswamy, Muruganantham Nithyanantham, P. S. Raghu

Departments of Phytopharmacy and Phytomedicine (TIFAC CORE HD), and Pharmaceutical Chemistry, JSS College of Pharmacy, Rocklands, Ooty, Tamilnadu, India

Submitted: 03-12-2011 Revised: 13-01-2012 Published: 22-11-2012

ABSTRACT

Background: *Nigella sativa* Linn (Ranunculaceae) is popularly known as black cumin with a wide spectrum of pharmacological activities including anti-inflammatory, antibacterial, antifungal and antihelmenthic. The seeds are externally applied for eruptions of skin. The seeds are used traditionally for psoriasis tropicus with general pain and eruption of patches. **Objective:** The ethanolic extract of *Nigella sativa* seeds were evaluated for antipsoriatic activity. **Materials and Methods:** The screening of antipsoriatic activity of 95% of ethanolic extract of *Nigella sativa* seeds by using mouse tail model for psoriasis and *in vitro* antipsoriatic activity was carried out by SRB Assay using HaCaT human keratinocyte cell lines. **Results:** The ethanolic extract of *Nigella sativa* seeds extract produced a significant epidermal differentiation, from its degree of orthokeratosis (71.36 ± 2.64) when compared to the negative control (17.30 ± 4.09%). This was equivalent to the effect of the standard positive control, tazarotene (0.1%) gel, which showed a (90.03 ± 2.00%) degree of orthokeratosis. The 95% ethanolic extract of *Nigella sativa* shown IC50 239 μg/ml, with good antiproliferant activity compared to Asiaticoside as positive control which showed potent activity with IC50 value of 20.13 μg/ml. **Conclusion:** The ethanolic extract of *Nigella sativa* seeds also showed increase in relative epidermal thickness when compared to control group by confirming its traditional use in psoriasis treatment.

Key words: Black cumin, mouse tail model, *Nigella sativa,* orthokeratosis, psoriasis

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.103650

Quick Response Code:

INTRODUCTION

Psoriasis is a chronic life-long inflammatory disease that primarily affects the skin, musculoskeletal system, the gastrointestinal system and the eye.[1] Being an autoimmune disorder no diagnostic tests available for identification of psoriasis.[2] However, the current treatments have not fully met the needs of the sufferers, largely due to the side effects so often associated with various therapies. Also, a large proportion of patients would develop drug resistance after long term drug exposure.[3] Natural remedies seem promising in the management of wide range of dermatological conditions including psoriasis vulgaris.[4]

Nigella sativa Linn. is an annual herb of the Ranunculaceae family. It is popularly known as black cumin. The *Nigella sativa* seeds contain ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids.[5-8] Pharmacological investigations of the seed extract reveal a wide spectrum of activities including anti-inflammatory,[9] antibacterial, antifungal and antihelmenthic.[10] The seeds are externally applied for eruptions of skin. The seeds are used traditionally for psoriasis tropicus with general pain and eruption of patches.[11] Although the seeds had been used for psoriasis, no scientific studies on this usage have yet been reported, so we have commenced a systematic study to assess the antipsoriatic activity of the ethanolic extract of *Nigella sativa* seeds evaluated using mouse tail model. Granular layer of the epidermis is greatly reduced in psoriatic lesions.[12] This condition is known as Parakeratotic condition which is seen in the adult mouse tail which is one of the characteristic features of psoriasis. Induction of Orthokeratosis in the adult mouse tail is the basis behind the mouse tail test.[13]

Many drugs presently used in the treatment of psoriasis have been evaluated by the mouse tail test and were found to have shown good efficacies.[14] Many herbs presently

Address for correspondence:
Dr. S. P. Dhanabal, Department of Phytopharmacy and Phytomedicine (TIFAC CORE HD), JSS College of Pharmacy, Post box No.20, Rocklands, Ooty, India.
E-mail: dhanabalsp@rediffmail.com

used in the treatment of psoriasis have been evaluated by this method and were found to have significant effects.[15] The *in vitro* antipsoriatic activity was carried out in HaCaT human keratinocyte cell lines.[16] It is a model of epidermal hyper proliferation in psoriasis we used a rapidly multiplying HaCaT human keratinocyte cell lines.[17] Hence, in this present study we evaluated 95% ethanolic extract of *Nigella sativa* seeds for psoriasis.

MATERIALS AND METHODS

Plant materials

Nigella sativa seeds were collected in the Mohali Region of the state of Punjab, India in the month of July 2009. The botanical identification was done by Dr. S. Rajan, Field Botanist, Central Council for Research in Homoeopathy, Emerald, Ooty, Nilgiris (District), Tamilnadu, India. Voucher specimens have been deposited at the Department of Phytopharmacy and Phytomedicine (TIFAC CORE HD), JSS College of Pharmacy, Rocklands, Ooty, India.

Extraction

The *Nigella sativa* seeds were washed with water to remove dust particles, Shade dried and extracted by boiling 500 g of the seed powder (twice) in 3000 mL of 95% Ethanol for 30 min at 70°C in a soxhlet extraction unit. The extract obtained was evaporated and concentrated on a water bath at atmospheric pressure to a semisolid condition, which was further dried in an oven at 30°C on a shallow dish to constant weight to remove the solvent completely (yield, 22.78%).

Phytochemical screening

Preliminary phytochemical analysis of the extract was performed by simple chemical tests.[18]

Cytotoxicity assay

Sulphorhodamine B Assay

Sulphorhodamine B (SRB) assay is used for *in vitro* antipsoriatic study.[16] HaCaT human keratinocyte cell lines were obtained from National Center for Cell Science, Pune, India. They were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The monolayer cell culture was trypsinized and the cell count adjusted to 1.0×10^5cells/ml using growth medium in a 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells/well) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once and 100 µl of drug dilution prepared in maintenance media was added per well in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25 µl of 50% trichloro-acetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form an overall concentration of 10%. The plates were then incubated at 4°C for one hour. The plates were flicked; culture was washed five times with tap water to remove traces of medium, drug and serum, and was then air dried. The air-dried plates were stained with SRB for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried.100 µl of 10 mM tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540 nm. Data obtained at different concentrations were used for IC50 calculations.

Pharmacological screening

Animals

Healthy male adult albino mice (25-30 g) obtained from the animal house of JSS College of Pharmacy, Ooty, Tamilnadu, India were used for the study. Mice were housed in polypropylene cages and fed on standard pellet diet and water ad libitum, and the room maintained under controlled condition (12 h light-dark cycle at 22±2°C). Animals were allowed to acclimatize for 7 days prior to experiments being carried out. Institutional ethics committee permission was obtained as per CPCSEA guidelines (Registration No: JSSCP/IAEC/M.PHARM/PHYTO PHARM/04/2009-2011) for carrying out the study in animals.

Mouse tail model

In vivo antipsoriatic activity was performed using mouse tail model for psoriasis.[13] Animals were separated in to three groups of six animals in each group. The first group served as control which was left untreated and the second group treated with standard tazarotene gel (0.1 %) which served as positive control. The third group was treated with 95% ethanolic extract of *Nigella sativa seeds*. The extract was dissolved with water in the ratio of 1:2 and used for the topical application on the tail especially at proximal part. Treatment was done for animals once daily for 14 days. 0.5 mL of the *Nigella sativa* seed extract or tazarotene was applied topically to the proximal part of the tail and allowed to be in contact with tail skin for 2 hours with the help of cloth plaster. The tails were washed with plain water at the end of contact time. The animals were sacrificed by deep ether anaesthesia, two hours after the last treatment and around 2.5 cm length the proximal parts of their tails were cut and they are preserved in different containers containing 10 % formalin in saline.

Histopathological evaluation

The tail skin was used to prepare longitudinal histological sections and subjected to staining using hematoxylin-eosin.

The tail specimens were analyzed histometrically for:

1. The horizontal length of an individual scale lying in between adjacent hair follicles including sebaceous glands (n=10 scales per animal, n=6 animals per treatment group; i.e. a total of 60 measurements per treatment)
2. The horizontal length of the fully developed granular layer within an individual scale (n=10 scales per animal, n=6 animals per treatment group; i.e. a total of 60 measurements per treatment), and
3. The vertical epidermal thickness between the dermo-epidermal junction and the lowest part of the stratum corneum (n=5 measurements per scale, n=10 scales per animal, n=6 animals per treatment group; i.e. a total of 300 measurements per treatment).

Taken together, from these calculations, the following three parameters were used for the evaluation of the drug effects: (a) the degree of orthokeratosis, (b) the 'drug activity' and (c) the relative epidermal thickness.

Drug Activity = OK(s)-OK(c)/100-OK(c)×100

with OK (i.e. orthokeratosis) as the mean of the parameter explained under for a test substance (s) and the untreated control condition (c), respectively.

Statistical analysis

Data in the present study are presented as weighed mean±standard error. For statistical comparisons in the mouse tail test explorative probabilities were obtained by the Mann Whitney U test. Statistical calculations were done using Graph Pad Prism software. Values with P<0.05 are considered significant.

RESULTS

The yield of ethanolic extract *Nigella sativa* was found to be 22.78%. The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, triterpenes, fats and oils, resins, phenols, tannins, flavanoids, proteins and aminoacids.

The ethanolic extract of *Nigella sativa* seeds produced significant differentiation in epidermis as seen from its degree of orthokeratosis (71.36±2.64%) when compared to control (17.30± 4.09%). It is equivalent to the standard tazarotene (0.1%) gel which showed 90.03±2.00% degrees of orthokeratosis. Overall, the *Nigella sativa* seed extract showed 65.01% activity in the mouse tail model for psoriasis [Figure 1 and Table 1]. In relative epidermal thickness, the 95% ethanolic extract of *Nigella sativa* showed significant increase when compared to control group.

The 95% ethanolic extract of *Nigella sativa* shown IC50 239 µg/ml, with good antiproliferant activity compared to Asiaticoside as positive control which shown potent activity with IC50 value of 20.13 µg/ml.

DISCUSSION

Due to disturbances in leukotriene homoeostasis can result in inflammatory responses as diverse as psoriasis, rheumatoid arthritis and inflammatory bowel disease. The presence of cysteinyl leukotrienes (slow-reacting substances) is reported in inflammatory diseases such as psoriasis.[19] It is well known underlying mechanisms of psoriasis. Since quercetin inhibit leukotriene synthesis and histamine release, as well as act as superoxide scavengers, they could have a palliative effect on inflammation.[20,21] Since flavonoids are well effective for psoriasis and our preliminary phytochemical investigation revealed the presence of flavanoids in ethanolic extract of *Nigella sativa* seeds, we carried out HPTLC analysis for estimating the presence of quercetin. The amount of quercetin was found to be 9.31%.

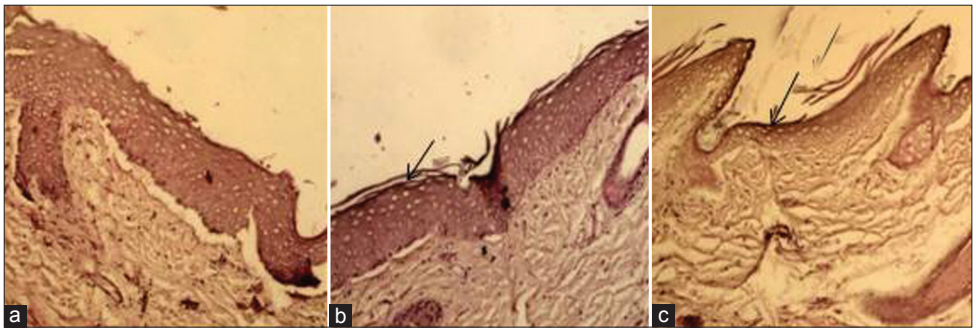

Figure 1: Histopathological sections of mouse tail skin treated topically for 14 days, (original magnification 40×) (a) Control; (b) Tazarotene 0.1%; (c) 95% ethanolic extract of Nigella sativa seeds. Note that granular layer is less developed in most parts of the control specimen (a), Tazarotene induced orthokeratosis are clearly seen over the whole horizontal length of the scale as black layer, marked with an arrow. (b), well developed granular layer is also seen in (c), which is treated with Nigella sativa seeds ethanolic extract

Table 1: Effects of 95% ethanolic extract of Nigella sativa seeds on the degree of orthokeratosis and relative epidermal thickness as well as the 'drug activity' in the mouse tail model

Treatment groups	Degree of orthokeratosis (%)	Drug activity (%)	Relative epidermal thickness (%)
Control	17.30±4.09*	0	100.00±10.7
Standard tazarotene (0.1%)	90.03±2.00*	87.94	103.56±4.7
Nigella sativa seed extract	71.36±2.64*	65.01	116.89

Values are expressed as mean±s.e.m., * $P<0.05$ with respect to control

The granular layer is present in epidermis. It is greatly reduced or almost absent in epidermis of psoriatic leisions.[12] This condition is known as parakeratosis, one of the most important characteristic features of psoriasis.[22] Granular layer formation around the epidermis is known as orthokeratosis condition. Conversion of parakeratosis condition to orthokeratosis is the main principle behind the mouse tail test.[13] Granular layer formation is indicated by the degree of orthokeratosis. The 95% ethanolic extract of *Nigella sativa* seeds produced well defined granular around the epidermis which is confirmed by its degree of orthokeratosis (71.36±2.64%).

Psoriasis is a disease resulted from the hyper proliferation and abnormal differentiation of keratinocytes.[23] A successful antipsoriatic drug that targets the epidermis is defined as a compound that ideally shows low toxicity and restores skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation, or both.[24] The present study aimed to investigate the anti-proliferative properties of 95% ethanolic extract of *Nigella sativa* seeds, which were selected from the most frequently used prescriptions in TCM for psoriasis treatment, for their anti-proliferative effects against keratinocytes using cultured HaCaT cells as a psoriasis-relevant experimental model.

Overall, the *Nigella sativa* seed extract showed 65.01% activity in the mouse tail model for psoriasis. In relative epidermal thickness, the 95% ethanolic extract of *Nigella sativa* showed significant increase when compared to control group.

CONCLUSION

From the present study it can be said that topical application of 95% ethanolic extract of *Nigella sativa* seeds has antipsoriatic activity and the external application is be beneficial in the management of psoriasis.

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Cite this article as: Dwarampudi LP, Palaniswamy D, Nithyanantham M, Raghu PS. Antipsoriatic activity and cytotoxicity of ethanolic extract of *Nigella sativa* seeds. Phcog Mag 2012;8:268-72.

Source of Support: Nil, **Conflict of Interest:** None declared.