ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 3, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF ANTIPSORIATIC ACTIVITY OF AQUEOUS EXTRACT OF *BRASSICA OLERACEA* VAR. *CAPITATA* AND ETHANOLIC EXTRACT OF *MENTHA SPICATA* LEAVES ON IMIQUIMOD-INDUCED PSORIASIS-LIKE DERMATITIS

AYESHA HUSNA S*, JAYASANKAR REDDY V

Department of Pharmacology, Krishna Teja Pharmacy College, Tirupati, Andhra Pradesh, India, Email: ayeshahusnas@yahoo.co.in

Received: 01 December 2018, Revised and Accepted: 21 January 2019

ABSTRACT

Objective: The objective of this study is to evaluate the antipsoriatic activity of aqueous extract of *Brassica oleracea* var. *capitata* leaves (BOAE) and ethanolic extract of *Mentha spicata* leaves (MSEE) on Imiquimod-induced Psoriasis-like dermatitis.

Methods: Imiquimod induction method for psoriasis-like dermatitis was used for the evaluation of antipsoriatic activity. Extracts were tested at a dose of 100 mg/kg body weight (b.wt.) and 400 mg/kg b.wt. in Swiss albino mice. These test doses were compared with the Retino-A (0.025%) treated group which serves as a standard group. Parameters studied in the test include changes in body weight, spleen weight and spleen index, neutrophils, lymphocytes, eosinophils, monocytes, hemoglobin, red blood cells, platelets, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, epidermal thickness, erythema, desquamation, skin thickness, superoxide dismutase, catalase, lipid peroxidation of skin and percentage orthokeratotic values, drug activity, and relative epidermal thickness.

Results: MSEE at dose of 400 mg/kg b.wt has shown more effect when compared with MSEE at low dose, BOAE topical and oral.

Conclusion: From the study, it was concluded that the above extracts have promising anti-psoriatic activity.

Keywords: *Brassica oleracea* var. *capitata*, *Mentha spicata*, Psoriasis, Imiquimod, Retino-A (0.025%).

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i3.30693

INTRODUCTION

Psoriasis is a condition in which the skin cells build up characterized by red skin, scales, and dry patches resulting in severe itching. It is a chronic, painful disease for which there is yet no cure. Psoriasis is a disorder of the skin, which occurs when the immune system sends out faulty signals, resulting in the speeding-up of the skin cell cycle [1,2]. It is a noncommunicable disease which can occur at any age. It affects patients physically as well as mentally. Psoriasis involves the skin and nails and is also associated with a number of comorbidities. The reported prevalence of psoriasis in countries ranges between 0.09% and 11.4%, making it a serious global problem. The etiology of psoriasis still remains unclear [3]. Although there is a reported suggestion that psoriasis could be an autoimmune disease, no autoantigen that could be responsible has been defined yet. In psoriasis epidermal hyperproliferation, abnormal keratinocyte differentiation can be seen. However, the cause of the loss of control of keratinocyte turnover is idiopathic. Early and active psoriatic lesions are characterized by intraepidermal penetration of activated polymorphonuclear leukocytes, which causes uncontrolled release of reactive oxygen species (ROS) which leads to pre-oxidative damage of skin membranes, which worsens the condition by increasing the severity of the lesions. ROS activates phospholipase A_2 and thus increase the release of the mediators of arachidonic acid. Prostaglandin produced by the cyclooxygenase pathway also contributes to psoriasis by dilating capillaries present in the dermis of the skin, stimulates keratinocyte cell growth and increase in leuckocyte infiltration [4].

Availability, affordability, side effects on prolonged usage of allopathic drugs have become a greatest concern for psoriasis. Hence, this study was aimed at assessing the antipsoriatic activity using herbal extracts of *Brassica oleracea* var. *capitata* and *Mentha spicata* leaves. These two plants are well-known plants. *B. oleracea* var. *capitata* is commonly known as cabbage and *M. spicata* plant is commonly known as spearmint. Both these plants are cultivated in India and has a wide usage. As *B. oleracea* var.*capitata* and *M. spicata* was used conventionally, but there is no reported data so an attempt was made to evaluate the antipsoriatic activity of *B. oleracea* var.*capitata* and *M. spicata* [5,6].

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *B. oleracea* var. *capitata* were collected from the local market. The leaves of the *M. spicata* plant were collected from the agriculture area near Tirupati in Chittoor District, AP, India. Both the plants were authenticated by Dr. K Madhava chetty, Assistant Professor, Department of Botany, S.V University, Tirupati. Then the plants were washed with distilled water to remove dirt and soil. Then, the collected *M. spicata* plant leaves were dried under shade at room temperature for 10–15 days. The dried plant leaves were powdered by using a grinding mill to obtain a coarse powder and then passed through 40 mesh sieve.

Preparation of plant extract (*Mentha spicata*)

The powdered material was subjected to extraction using ethanol by the solvent extraction method. Initially, 100 g of crude powder was taken and packed in a packing paper. This pack was placed in a Soxhlet extractor and extracted with ethanol, the extraction was carried out until the extract becomes colorless. The extract was then filtered with what man filter papers (No.1) and the filtrate was evaporated to dryness in a rotary evaporator at 40° [4]. The obtained crude extract was stored in a refrigerator at 4°C until the time of use.

The percentage yield of the extract was calculated by using the following formula

$$\%\,\text{yield} = \frac{\text{weight of the extract}}{\text{weight of the plant material}} \times 100$$

Preparation of plant extract (*Brassica oleracea* var.*capitata*)

The fresh leaves of *B. oleracea* var.*capitata* were collected and washed thoroughly under running water. Then, the leaves were blended with a blender. After that, the blended plant material was pressed to get the pure extract of *B. oleracea* var.*capitata*. This extract was treated with Kaolin. Then, the extract was concentrated on the hot plate. The pure organic part of the sample was thus prepared [7] and stored at 4–5°C. This final extract was redissolved in sterile water before experimentation for the evaluation of the antipsoriatic activity.

Table 1: The detected compounds in both the plant extracts

Name of the test	Results	
	MSEE	BOAE
Alkaloids	+ve	+ve
Carbohydrates	+ve	+ve
Proteins	+ve	+ve
Phenols	+ve	+ve
Flavanoids	+ve	+ve
Amino acids	+ve	+ve
Glycosides	+ve	+ve
Saponins	+ve	-ve
Terpenes	-ve	+ve
Tannins	+ve	+ve

+ve: Presence of compounds, -ve: Absence of compounds. MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

PRELIMINARY PHYTOCHEMICAL SCREENING

The standard screening test of the extract was performed for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures [8,9] [Table 1].

ACUTE ORAL TOXICITY

An acute toxicity study was carried out as per OECD-423 guidelines.

ANTIPSORIATIC ACTIVITY

All experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee (IAEC) and Committee for the purpose of control and supervision of Experiments

Table 2: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on weight parameters in imiquimod induced psoriasis

Weight parameters	Normal	Control	Standard	BOAE		MSEE	
				Topical	Oral	Low	High
Body weight	24.25±1.1	17.4±0.98	23.0±0.85	19.4±1.06	20.95±1.04	20.2±1.5	21.8±1.3
Spleen weight	1.2±0.49	5.1±1.46	2.51±0.93	4.47±1.03	3.18±0.650	2.84±1.0	2.9±1.4
Spleen index	5.28±0.925	16.65±0.89	5.94±1.32	12.00±0.56	9.5±0.94	10.6±1.3	6.2±1.0

All values were expressed as mean±SEM, (n=6).Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 3: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on heamotological parameters in imiquimod induced psoriasis

Heamotological parameters	Normal	Disease	Standard	BOAE		MSEE	
				Topical	Oral	Low dose	High dose
Neutrophils (%)	13.5±4.1	7.26±4.1	11.3±4.68[b]	8.1±4.1[c]	9.2±3.4[c]	8.6±5.2[b]	10.4±3.9[c]
Lymphocyte (%)	62.2±4.14	91.6±4.8	67.0±5.7[c]	85.6±3.7[c]	75.2±9.5[b]	81.8±7.5[c]	71.0±11.9[b]
Eosinophils (%)	2.7±1.78	7.3±1.2	2.2±1.3[c]	4.6±1.3[c]	4.0±1.5[b]	6.2±1.10[c]	2.2±1.3[c]
Monocyte (%)	2.12±1.60	7.0±1.5	1.9±1.43[b]	4.2±1.48[c]	2.2±1.3[c]	4.0±1.58[b]	1.84±1.35[c]
Hemoglobin (g/dl)	11.31±0.5	7.89±0.62	10.79±0.53[c]	9.38±0.53[b]	10.16±0.53[b]	9.66±0.49[c]	10.36±0.4[c]
RBC (milli/cumm)	4.4±0.7	2.11±0.61	3.97±0.47[c]	2.78±0.40[c]	3.7±0.5[b]	3.28±0.68[a]	3.61±0.3[c]
Platelets (lakhs/cumm)	2.65±0.4	1.65±0.3	2.51±0.65[a]	1.94±0.4[c]	2.21±0.4[c]	2.00±0.5[b]	2.31±0.40[c]
PCV	15.26±1.3	31.8±1.6	17.0±1.2[c]	23.4±1.83[b]	19.2±1.56[c]	21.8±1.6[b]	17.9±1.6[c]
MCV	43.35±1.03	54.8±1.5	44.9±1.04[c]	52.5±1.5[c]	49.3±1.45[b]	51.0±1.4[b]	46.2±1.0[c]
MCH	14.86±0.89	23.3±1.6	17.4±1.5[c]	22.1±1.3[b]	19.3±0.7[c]	21.3±0.8[c]	17.9±0.9[b]
MCHC		26.6±1.0	32.1±2.1[c]	27.0±0.8[c]	30.3±1.8[a]	29.0±1.3[b]	32.2±1.2[c]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cells, SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 4: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on skin in imiquimod induced psoriasis

Skin parameters	Normal	Disease	Standard	BOAE		MSEE	
				Topical	Oral	Low	High
Orthokeratosis (%)	62.3±1.2	12.18±2.02	60.04±2.3[c]	19.63±2.9[b]	40.6±2.2[c]	32.8±2.8[b]	55.4±1.9[c]
Drug activity (%)	-	-	54.01	5.18	24.31	18.08	32.1
Relative epidermal thickness (%)	71.4±4.6	100.0±9.02	67.5±5.18[c]	52.3±9.65[a]	56.9±4.0[c]	54.3±2.7[c]	65.87±3.7[b]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 5: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on erythema in imiquimod induced psoriasis

Erythema	Disease	Standard	BOAE		MSEE	
			Topical	Oral	Low	High
On 7th day	4.0±0.4	3.9±0.2[b]	4.2±0.3[b]	4.6±0.3[b]	3.7±0.2[c]	4.3±0.16[c]
On 12th day	4.5±0.2	2.4±0.1[c]	3.6±0.2[c]	2.4±0.27[b]	3.0±0.19[b]	2.4±0.2[b]
On 21th day	5.2±0.17	1.8±0.15[c]	2.9±0.3[a]	2.3±0.18[b]	2.9±0.14[c]	1.02±0.12[c]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 6: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on desquamation in imiquimod induced psoriasis

Desqumation	Disease	Standard	BOAE		MSEE	
			Topical	Oral	Low dose	High dose
On 7th day	4.0±0.4	3.9±0.2[c]	4.2±0.3[a]	4.6±0.3[b]	3.7±0.2[c]	4.3±0.16[c]
On 12th day	4.5±0.2	2.4±0.1[c]	3.6±0.2[b]	2.4±0.27[a]	3.0±0.19[c]	2.4±0.2[b]
On 21th day	5.2±0.17	1.8±0.15[c]	2.9±0.3[c]	2.3±0.18[b]	2.9±0.14[c]	1.02±0.12[c]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]P<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 7: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on skin thickness in imiquimod induced psoriasis

Skin thickness	Normal	Disease	Standard	BOAE		MSEE	
				Topical	Oral	Low dose	High dose
On 7th day	0.12±0.01	1.51±0.11	1.43±0.11[c]	1.97±0.3[a]	2.01±0.1[c]	1.89±0.3[a]	2.4±0.3[b]
On 12th day	0.14±0.02	1.87±0.12	1.01±0.1[c]	1.8±0.1[c]	1.4±0.29[a]	1.63±0.1[c]	1.2±0.2[b]
On 21st day	0.11±0.03	2.03±0.2	0.39±0.1[c]	1.5±0.134[c]	1.2±0.2[b]	1±0.1[c]	1.01±0.1[c]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 8: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on epidermal thickness in imiquimod induced psoriasis

Epidermal thickness	Disease	Standard	BOAE		MSEE	
			Topical	Oral	Topical	Oral
On 7th day	4.3±0.34	4.0±0.45[b]	4.8±0.57[a]	4.2±0.78[a]	4.9±0.56[b]	3.9±0.34[b]
On 12th day	4.5±0.46	2.10±0.32[c]	4.3±0.34[b]	3.8±0.44[b]	4.0±0.60[a]	3.5±0.56[b]
On 21st day	4.8±0.54	1.10±0.22[c]	3.5±0.53[b]	2.75±0.46[a]	3.1±0.35[b]	1.54±0.23[c]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

Table 9: Effect of aqueous extract of *Brassica oleracea* var. *capitata* leaves and ethanolic extract of *Mentha spicata* leaves on antioxidants in Imiquimod induced psoriasis

Antioxidant parameters	Normal	Disease	Standard	BOAE		MSEE	
				Topical	Oral	Low dose	High dose
SOD (units/mg)	20.24±0.932	8.71±0.843	18.18±1.01[c]	12.68±1.8[a]	13.48±1.2[b]	14.98±0.943[c]	16.24±0.98[b]
CAT (units/mg)	6.85±0.6	2.01±0.932	4.63±0.683[c]	3.69±0.915[b]	4.02±0.74[c]	3.87±0.98[a]	4.29±0.87[b]
LPO (n.mol/mg)	2.09±0.587	15.31±0.73	4.83±0.673[c]	4.4±0.88[a]	3.84±0.72[b]	4.2±0.62[b]	3.86±0.54[c]

All values were expressed as mean±SEM, (n=6). Statistical significance: [a]p<0.05, [b]p<0.01, [c]p<0.001 were compared with disease control (one-way ANOVA followed by Dunnet's multiple comparision test). SEM: Standard error of mean, MSEE: Ethanolic extract of *Mentha spicata* leaves, BOAE: Aqueous extract of *Brassica oleracea* var. *capitata* leaves

on Animals (CPCSEA) rules were in accordance with the guidelines of IAEC (Regd.No.1521/PO/a/11/CPCSEA). The anti-psoriatic activity of plant extracts was reviewed through different tests [Tables 2-9]. Male Swiss mice (weighing 20–30 g) at the age of 8 to 11 weeks were used for the study. Animals were allowed for free access to water and standard chow diet up to the end of the 23-day experimental period.

The animals were divided into seven groups. Each group consists of five mice. All the animals were shaved on the back using hair removal cream (Veet). After 24 h of hair removal, 62.5 mg of Imiquimod (translating 3.125 mg of active compound) was applied on the shaved back using the applicator brush for 7 consecutive days for all the animals except for normal group [10].

- Group-I: Normal control group of mice received normal saline and regular feed
- Group-II: Disease control group of mice. This group of animals was induced with Imiquimod 5% cream at a dose of 62.5 mg (translating 3.125 mg of active compound) on the shaved back topically for 7 consecutive days. Mice were given acetate in drinking water for 7 days to enhance Imiquimod-induced skin inflammation [11]
- Group-III: Standard control group of mice was induced with psoriasis same as Group-II animals. On the 8th day, Retino-A 0.025% was applied topically for the other 16 days [12]
- Group-IV: This group was induced with psoriasis same as Group-II animals. On the 8th day, *B. oleracea* var.*capitata* extract was diluted with distilled water and given orally at a dose of 400 mg/kg b.wt. for 16 days
- Group-V: This group was induced with psoriasis same as Group-II animals. On the 8th day, *B. oleracea* var.*capitata* extract was applied topically as a 0.4% concentration for 16 days
- Group-VI: This group was induced with psoriasis same as Group-II animals. On the 8th day, *M. spicata* extract was diluted with distilled water and given orally at a dose of 200 mg/kg b.wt. for 16 days
- Group-VII: This group was induced with psoriasis same as Group-II animals. On the 8th day, *M. spicata* extract was diluted with distilled water and given orally at a dose of 400 mg/kg b.wt. for 16 days.

On every alternate day, the thickness of skin and ear thickness was measured using a digital micrometer (SLB Works). The increase in skin thickness was taken as a measure of skin inflammation [13].

Scoring severity of inflammation

The severity of inflammation on the back skin- an objective scoring system was developed based on the clinical Psoriasis Area and Severity Index (PASI). This is an online application. Erythema, induration, and desquamation were scored independently on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked.

Assessment of body weight

Body weight of the animals is assessed before the experiment and on the last day after 2 h of treatment, animals were weighed, and weight was recorded [14].

Sample collection

At the end after 2 hs of final treatment mice were weighed then

- Blood was collected through retro-orbital route
- Then, animals were sacrificed by deep ether anesthesia, and back skin of mice was collected
- Then, the spleen was isolated.

Histopathological examination of skin

The skin that was isolated was stored in separate containers containing 10% formalin in saline. Longitudinal histology sections of animal skin were and stained with hematoxylin-eosin [15,16].

Changes in spleen

The spleen from each mouse was isolated and was weighed. Splenomegaly was evaluated by calculating the ratio of the weight of the spleen to the body weight [17].

The spleen index is calculated using formula,

$$\text{Spleen index} = \text{spleen weight}/\text{body weight} \times 100$$

Biochemical parameter estimations

Skin tissues were collected from the experimental animals, and the tissues were homogenized to carry out the *In vivo* antioxidant parameters. The antioxidant studies that were carried out are lipid peroxidation (LPO) [18], catalase (CAT) [19], and superoxide dismutase [20].

Hematological parameters

Blood was collected through retroorbital route and stored in K_2 ethylenediaminetetraacetic acid vial for the complete blood count. Blood was analyzed for the following parameters as hemoglobin (Hb %), red blood cell (RBC), lymphocytes, neutrophils, eosinophil, monocyte, platelet count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC) were estimated with the help of hematology analyzer (Medonic CA620, Boule, Sweden).

Statistical analysis

The results are expressed as mean±standard error of the mean. Comparison between the treatment groups and control were performed using Graph pad prism06 version by ANOVA method followed by multiple comparisons of Dunnet's method.

RESULTS

Effect of IMQ-induced psoriasis in mice

Morphological findings

Scales and redness on the skin of mice was observed after application of Imiquimod

Assessment of psoriasis using psoriasis area and severity index

Morphological characters and health status, such as water and food consumption, behavior signs, body weight, cardiovascular signs, and respiratory patterns of all mice were normal throughout the experimental period. Two or three days after starting the IMQ application, it was observed that the dorsal skin exhibited signs of erythema, scaling, and thickening (Fig. 2). Thereafter, the intensity of psoriasis-like symptoms of diseased mice progressively increased in severity until the end of the treatment (day 16). However, mice in Group I treated daily with Vaseline did not show any signs of inflammation on the dorsal skin (Fig. 1). The independent PASI scores showed the continuous increase in levels of inflammation after IMQ application from day 1 to day 7, before initiation of treatment with Retino-A, aqueous extract of *B. oleracea* var. *capitata* (BOAE), ethanolic extract of *M. spicata* (MSEE). The intensity of PASI scores reached a peak on the seventh day after IMQ treatment which indicates successful induction of psoriasis-like dermatitis in the IMQ-treated mice.

However, there was a statistically significant decrease in psoriasis-like symptoms beginning on day 9, the 2nd day after initiation of treatment with Retino-A (group III), BOAE (groups IV, V), MSEE (Groups VI, VII). These symptoms consistently declined until day 16. The individual

Fig. 1: Normal

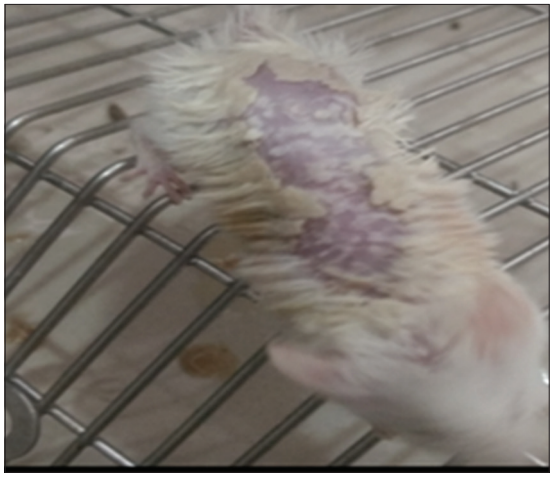

Fig. 2: Diseased

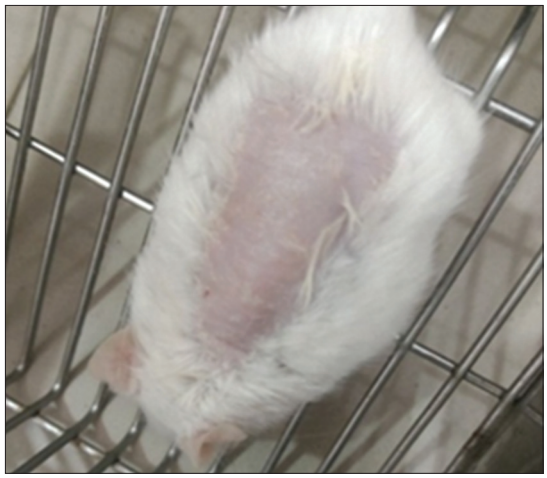

Fig. 3: Standard

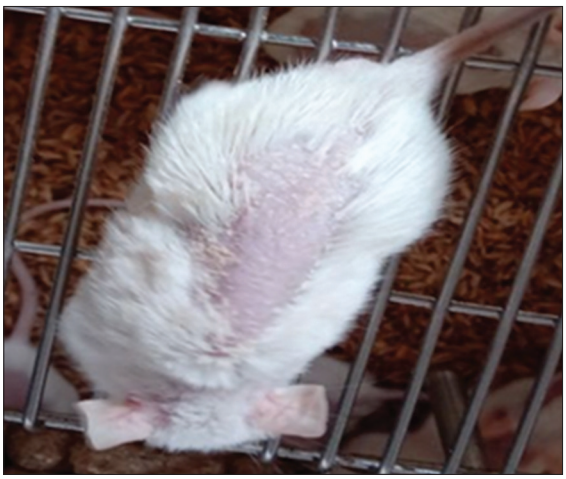

Fig. 4: Aqueous extract of *Brassica oleracea* var. *capitata* topical

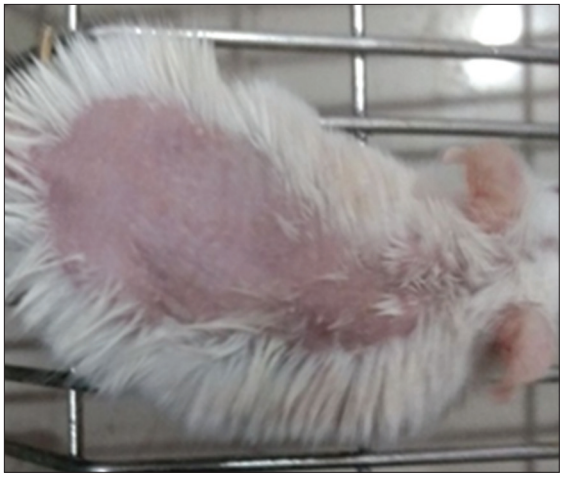

Fig. 5: Aqueous extract of *Brassica oleracea* var. *capitata* oral

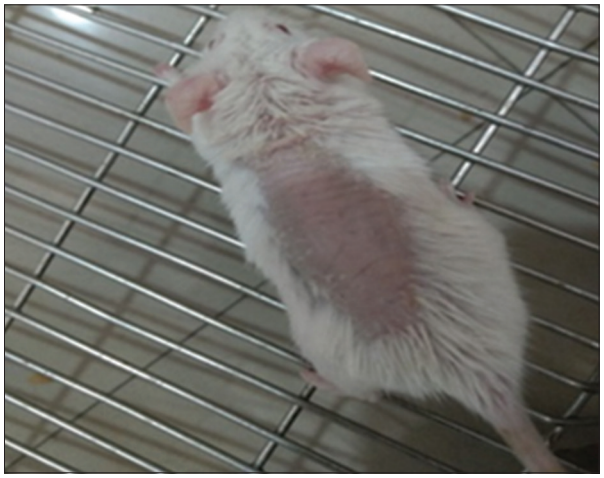

Fig. 6: Ethanolic extract of *Mentha spicata* low dose

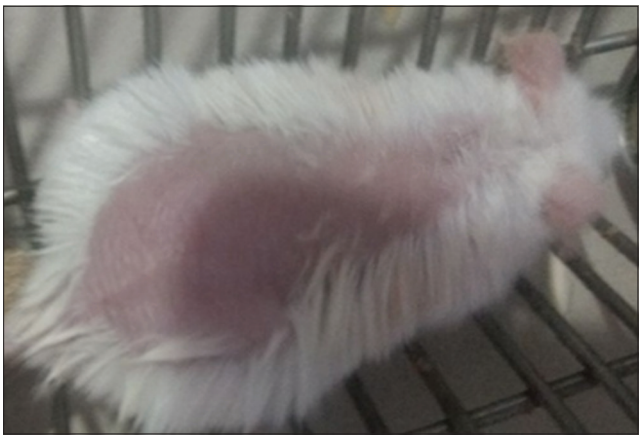

Fig. 7: Ethanolic extract of *Mentha spicata* high dose

PASI scores and the cumulative scores of all groups from days 1 to 16 are depicted in Fig. 1-7. Comparing with the IMQ-treated group, BOAE, MSEE treated groups showed a significant inhibitory effect on IMQ-induced psoriasis-like dermatitis. The route-dependent reduction in the PASI inflammatory symptoms was observed in the BOAE -treated groups at doses of 400 mg/kg b. wt. and dose-dependent reduction PASI inflammatory symptoms were observed in the BOAE -treated group at dose administrations of 100 mg/kg b.wt., 400 mg/kg b.wt. of mice. The marked reduction of PASI scores of Retino-A treated group was comparable to the 400 mg/kg b.wt. treated group.

Histological slides

The histopathology slides helped in evaluating different parameters like inflammatory cells, neutrophils, etc,. [Fig.8] [34].

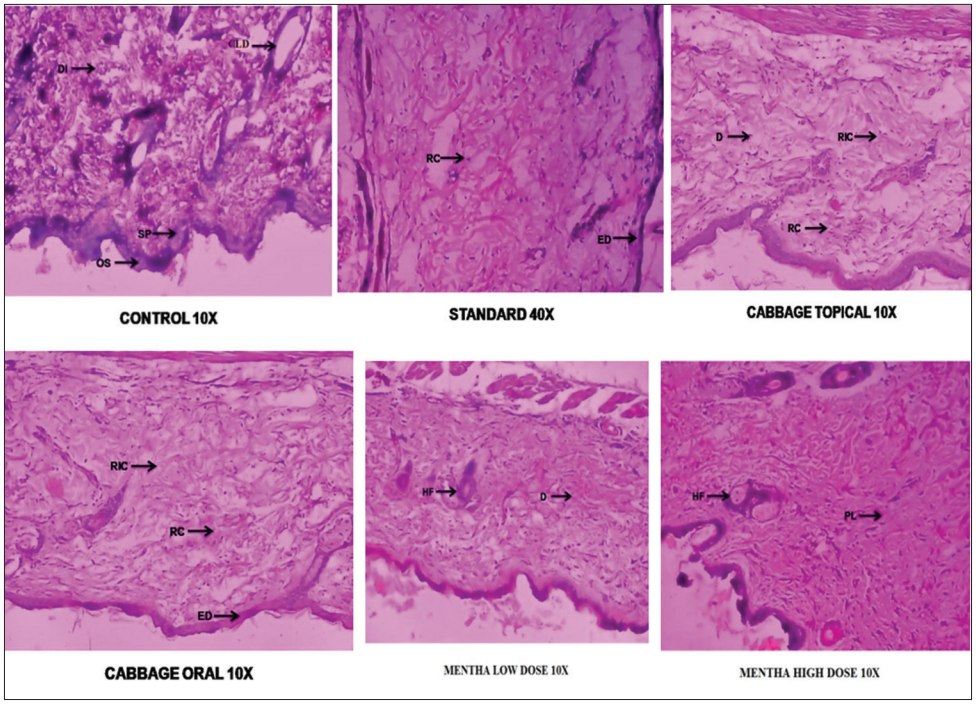

Fig. 8: Histopathological features on imiquimod induced psoriasis in aqueous extract of *Brassica oleracea* var. *capitata* and ethanolic extract of *Mentha spicata* treated rats. D: Dermal layer, DC: Degenerative changes, DI: Dermal inflammatory cells, ED: Epidermis, HF: Hair follicle, OS: Orthokeratosis, PL: Papillary layer, RC: Regenrative changes, RIC: Reduced Inflammatory cells, SP: Spongiotic sites, CLD: Capillary loop dilation

DISCUSSION

Swiss albino male mice were used in this experiment due to its better tolerance level to Imiquimod compared to female [10]. As the autoantigen causing psoriasis is not known, there is no permanent cure for this disease. The medical care whichever available in the market is treating the disease temporarily.

Natural plant ingredients are often used as novel therapeutic agents to control the inflammation with reducing side effects. As *B. oleracea* var. *capitata* and *M. spicata* was used traditionally, but there is no reported data so an attempt was made to evaluate the antipsoriatic activity of *B. oleracea* var.*capitata* and *M. spicata* [5,6].

Morphological findings and calculating PSAI confirms the attack of psoriasis-like dermatitis [21]. The decrease of the spleen weight/body weight ratio and cellularity of PALS after treatment with BOAE,MSEE in the IMQ-induced psoriasis-like dermatitis mice may be suggestive of a deficit in T-independent humoral immune responses [22]. Low number of neutrophils in the blood indicates the presence of infection [23,24]. Increase in lymphocytes that the immune system is working heavily, which is resulting in hyperproliferation of skin cells [25]. Eosinophils provide inflammatory signals that accelerate the pathogenesis of psoriasis [26]. High levels of monocytes indicate autoimmune disorders [27]. In auto-immune diseases, the immune system attacks and destroys RBC, platelets [28] and platelets may get trapped in the spleen [29]. PCV, MCV, MCH, and MCHC results indicate deranged pattern of iron status [30]. The decrease in the degree of orthokeratosis indicates destruction of granular layer [31]. Increase in erythema, desquamation, and skin thickness shows the presence of psoriasis morphologically [21]. If oxidative stress is persisting, or its level very high, the protein damage became profound and a decreased superoxide dismutase, CAT activity may occur [32]. LPO is a useful marker of oxidative stress because it is linked to increased production of ROS [33]. The histological studies reveal that the skin section treated/induced with Imiquimod showed a degenerative changes in skin tissue shows thickened epidermis, increased keratinization decrease in percentage of orthokeratotic regions, elongation of rette ridges, capillary loop dilation with minimal grade lesion of Munro 's microabcess, vacuolization which indicates the severe damage of skin tissue. Animals treated with Retino-A and BOAE, MSEE shows normal cytoarchitecture of skin tissue indicates regenerative changes [34].

CONCLUSION

This study showed that the aqueous extract of *B. oleracea.* var. *capitata* and ethanolic extract of *M. spicata* leaves exhibits a significant antipsoriatic activity. Route of administration and also Dose dependently decreased the relative epidermal thickness of animal skin as well as other histopathology features. Hematological parameters have also shown that these plant extracts play a prominent role in the recovery of psoriatic-like symptoms. The study implies that *B. oleracea e* var.*capitata* and *M. spicata* leaves could be used as natural therapeutic drugs to prevent complications related to psoriasis and authentifies the folk claim of the plant in the use of traditional medicine for the treatment of psoriasis. Additional clinical investigation of these extracts is needed to evaluate the efficacy and safety of their application as dietary supplements with health benefits to psoriasis patients. Further molecular level investigation is needed to prove its antipsoriatic activity.

ACKNOWLEDGEMENT

I express my profound thanks to my Father Syed Jowhar Madani & my Mother S. Sharmila for financial and moral support. I extend my thanks to all my friends who helped me to carry out the experiment. We extend our sincere gratitude to Dr. V. Jayasankar Reddy, HOD of Department of Pharmacology and Dr. P. Jayachandra Reddy, Principal, Krishna Teja Pharmacy College,Tirupati, India for providing instrumental facilities and guidance.

AUTHOR' S CONTRIBUTION

S. Ayesha Husna reporting anti-psoriatic activity of aqueous extract of *B. oleracea* var. *capitata* and ethanolic extract of *M. spicata* leaves

on imiquimod induced psoriasis like dermatitis,preparation of the manuscript,Dr. V. Jayasankar Reddy supervised the work.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Azfar RS, Gelfand JM. Psoriasis and metabolic disease: Epidemiology and pathophysiology. Curr Opin Rheumatol 2008;20:416-22.
2. Abdelgawad R, Nasr M, Hamza MY, Awad GA. Topical and systemic dermal carriers for psoriasis. Int J Curr Pharm Rev Res 2016;8:4-9.
3. Bowcock AM, Cookson WO. The genetics of psoriasis, psoriatic arthritis and atopic dermatitis. Hum Mol Genet 2004;13:R43-55.
4. Amigó M, Payá M, De Rosa S, Terencio MC. Antipsoriatic effects of avarol-3'-thiosalicylate are mediated by inhibition of TNF-alpha generation and NF-kappaB activation in mouse skin. Br J Pharmacol 2007;152:353-65.
5. Psoriasis Revolution. Psoriasis Cure-How to Cure Psoriasis 100% Naturally; 2017. Available from: https://www.youtube.com.
6. Ashley Marcin. Can You Use Oils to Treat Psoriasis? 2018. Available from: https://www.healthline.com/health/essential-oils-for-psoriasis.
7. Mandloi S, Mishra R, Yadav N, Yadav S. Antibacterial activity of *Mentha spicata* ethanol leaf extract against *Pseudomonas aeruginosa* isolated from upper respiratory tract of T.B. negative patients. World J Pharm Pharm Sci 2016;6:837-47. 5. Lewis JJ. Cabbage extracts and insulin-like activity. Br J Pharmacol Chemother 1950;5:21-4.
8. Ahmed MF, Rao AS, Ahmed SR, Ibrahim M. Phytochemical studies and antioxidant activities of *Brassica oleraceae*. L. var.Capitata. Int J Pharm Pharm Sci 2012;4:374-8.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Vol. 40. Pune: Nirali Prakashan; 2007. p. 1-635.
10. van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, *et al.* Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol 2009;182:5836-45.
11. Muruganantham N, Basavara KH, Dhanabal SP, Praveen T, Shamasundar NM, Rao KS. Screening of *Caesalpinia bonduc* leaves for antipsoriatic activity. J Ethnopharmacol 2011;133:897-91.
12. Shrivastav S, Sindhu RK, Kumar S, Kumar P. Anti-psoriatic and phytochemical evaluation of *Thespesia populnea* bark extracts. Int J Pharm Pharm Sci 2009;1:176-85.
13. Nadeem A, Ahmad SF, Al-Harbi NO, El-Sherbeeny AM, Al-Harbi MM, Almukhlafi TS, *et al.* GPR43 activation enhances psoriasis-like inflammation through epidermal upregulation of IL-6 and dual oxidase 2 signaling in a murine model. Cell Signal 2017;33:59-68.
14. Swindell WR, Michaels KA, Sutter AJ, Diaconu D, Fritz Y, Xing X, *et al*. Imiquimoid has strain-dependent effects in mice and does not uniquely model human psoriasis. Genome Med 2017;9:1-21.
15. Nakaguma H, Kambara T, Yamamoto T. Rat ultraviolet ray B photodermatitis: An experimental model of psoriasis vulgaris. Int J Exp Pathol 1995;76:65-73.
16. Singhal M, Kansara N. *Cassia tora* Linn cream inhibits ultraviolet-B-induced psoriasis in rats. ISRN Dermatol 2012;2012:346510.
17. Grine L, Steeland S, Van Ryckeghem S, Ballegeer M, Lienenklaus S, Weiss S, *et al*. Topical imiquimod yields systemic effects due to unintended oral uptake. Sci Rep 2016;6:1-6.
18. Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J 2013; 21:143-52.
19. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
20. Buege J, Aust SD. Microsomal lipid peroxidation. In: Colowick SP, Kaplan NO, editors. Methods in Enzymology. New York: Academic Press; 1978. p. 302-11.
21. Al-Mosawi AM. Antipsoriatic activity of oral and topical milk thistle extract against imiquimod-induced psoriasis-like skin lesions in balb/c mice. J Int Acad Res Multidiscip 2016;4:1-10.
22. Na Takuathung M, Wongnoppavich A, Panthong A, Khonsung P, Chiranthanut N, Soonthornchareonnon N, *et al*. Antipsoriatic effects of wannachawee recipe on imiquimod-induced psoriasis-like dermatitis in BALB/c mice. Evid Based Complement Alternat Med 2018;2018:7931031.
23. Hu SC, Yu HS, Yen FL, Lin CL, Chen GS, Lan CC. Neutrophil extracellular trap formation is increased in psoriasis and induces human β-defensin-2 production in epidermal keratinocytes. Sci Rep 2016;6:3119.
24. Barbara EO. Psoriasis it is not. J Clin Rheumatol 2013;19:296.
25. Langewouters AM, van Erp PE, de Jong EM, van de Kerkhof PC. Lymphocyte subsets in peripheral blood of patients with moderate-to-severe versus mild plaque psoriasis. Arch Dermatol Res 2008;300:107-13.
26. Kim HJ, Roh JY, Jung Y. Eosinophils accelerate pathogenesis of psoriasis by supporting an inflammatory milieu that promotes neutrophil infiltration. J Invest Dermatol 2018;138:2185-94.
27. Golden JB, Groft SG, Squeri MV, Debanne SM, Ward NL, McCormick TS, *et al.* Chronic psoriatic skin inflammation leads to increased monocyte adhesion and aggregation. J Immunol 2015; 195:2006-18.
28. Berentsen S, Sundic T. Red blood cell destruction in autoimmune hemolytic anemia: Role of complement and potential new targets for therapy. J Biomed Biotechnol 2015;363278:1-11.
29. Harry S. Jacob. Enlarged spleen (Splenomegaly); 2018. Available from: https://www.msdmanuals.com>home>blood disorders>spleen disorders.
30. Ponikowska M, Tupikowska M, Kasztura M, Jankowska EA, Szepietowski JC. Deranged iron status in psoriasis: The impact of low body mass. J Cachexia Sarcopenia Muscle 2015;6:358-64.
31. Bosman B, Matthiesen T, Hess V, Friderichs E. A quantitative method for measuring antipsoriatic activity of drugs by the mouse tail test. Skin Pharmacol 1992;5:41-8.
32. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the in the entire antioxidant defence grid. Alexandria Med J 2017;2017:1-7.
33. Ferretti G, Bacchetti T, Campanati A, Simonetti O, Liberati G, Offidani A, *et al.* Correlation between lipoprotein(a) and lipid peroxidation in psoriasis: Role of the enzyme paraoxonase-1. Br J Dermatol 2012;166:204-7.
34. Vijayalakshmi A, Geetha M. Anti-psoriatic activity of flavonoids from *Cassia tora* Leaves using the rat ultraviolet B ray photodermatitis model. Rev Bras Farmacogn 2014;24:322-9.

Author Query???
AQ1: Kindly note in your given word file the reference number 7 and 5 seems to be combined as single reference. We highlight that reference. So, please check and advice what we do.