Preventive and therapeutic effects of aqueous extract of *Spinacia oleracea* on Psoriatic patches in albino rats

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**Abstract**- Psoriasis has become a topic of global concern because of consistency in its prevalence according to Global Report on Psoriasis 2016, however, till date, no therapy has provided complete cure of this disease and no useful measure was discovered to prevent it or eliminate the risk of its relapse. Hence, the present study was designed to evaluate anti-psoriatic effects of *Spinacia oleracea* due to its anti-inflammatory, anti-oxidant, anti-proliferative and skin-strengthening contents. Psoriasis was induced by oral potassium iodide solution which was then determined by two methods i.e. percentage reduction in psoriatic patch size and psoriasis area and severity index (PASI) score to measure the decrease in severity. Twenty albino rats were used in each method, grouped as standard, curative, preventive and control with five rats in each group. Therapeutic and preventive doses for *S. oleracea* in both the methods were calculated according to daily intake guidelines of National Cancer Institute, USA, and German Nutrition Society, DGE guidelines respectively with slight modification. Methotrexate was used as standard drug along with folic acid to avoid toxic effects. This study reveals that *S. oleracea* has both therapeutic and preventive effects. It may be concluded that *S. oleracea* can be effectively used as sole therapy for psoriasis.

**Keywords**: Psoriasis, *Spinacia oleracea*, erythema, induration, desquamation.

**INTRODUCTION**

Psoriasis is a chronic, inflammatory, non-contagious and painful disease-carrying state with an upward trend of prevalence worldwide that varies between 0.09% and 11.4% (Gibbs 1996; Danielson et al., 2013). It affects quality of life resulting in psychological & mental distress, social, emotional and physical disabilities along with comorbidities such as depression, cardiovascular diseases, and arthritis. World Health Organization gave prime importance to psoriasis and issued a Global Report on it in 2016. Public awareness programs have been developed by World Health Organization (WHO), Centers for Disease Control & Prevention (CDC) and European Academy of Dermatovenerology (EADV) to aware the population about this disease since its treatment is mainly symptomatic including lifelong topical, systemic and photo-therapies but to date no therapy has been entirely successful in cure of this disease and ruling out the risk of relapse.

Uncertainty still lies in the etiology of psoriasis but as studies revealed, it is mainly inherited however non-inherited factors also provoke this disorder serving as precipitating elements or triggers in pre-disposed individuals (Wahab et al., 2012). Immune system sends wrong signals causing skin cells to grow quicker than normal cell division and as newly formed skin cells mature and come over the surface of previously formed skin cells, they give an appearance of raised patches (NIH, 2016). Causes may include the combination of genetic predisposition which may be triggered by factors such as stress, skin injury, smoking, alcohol, certain infections e.g., pharyngitis, HIV and strep throat, some drugs e.g. anti-malarial, lithium, beta blockers, and NSAIDs. Clinically, psoriasis may be plaque, guttate, flexural, pustular, erythrodermic, scalp, psoriatic arthropathy, psoriatic nail or palmoplantar type depending upon symptoms and areas of the body affected (NPF, 2016). An increased concentration of nitric oxide and hydroxyl radicals have been reported by few researchers in the skin of psoriatic patients suggesting that it may be due to low intake of anti-oxidants or is an inflammatory disorder that needs anti-inflammatory as well as anti-oxidant and anti-proliferative treatments (Declercq and Pouliot, 2013). The National Institute for Health and Care Excellence (NICE) guidelines suggest topical agents as first-line therapy for the treatment of psoriasis. Phototherapy and non-biologic systemic agents as second-line therapy while systemic biologic agents as the third line of treatment depending upon patient’s condition (NIH Clinical Guidelines 2012).

Various studies report the presence of carotenoids such as beta-carotene, lutein, zeaxanthin, astaxanthin, neoxanthin, violaxanthin and flavonoids such as spinacetin, patuletin, and jaceidin in *S. oleracea*. These constituents and other phyto-chemicals of *S. oleracea* are thought to possess anti-oxidant and anti-inflammatory activities that are required to maintain healthy skin and preserve normal functions of the body by preventing disease onset. *S. oleracea* also contains vitamin A, vitamin B, vitamin C, folate and trace elements that combat various skin abnormalities. There are nitrates, diacylglycerols, omega-
3-fatty acids and other phytochemicals (quercetin) which serve as precursors for the synthesis of anti-inflammatory mediators (Aehle et al 2004; Garg et al 2010; Otari et al 2010; Nagar et al 2011; Jaime et al 2015; Rahati 2015; Roberts and Moreau 2016).

Psoriasis is an inflammatory skin disorder that may involve nails, joints and various other body parts, hence this study was designed to evaluate anti-psoriatic effects of S. oleracea in rats due to its anti-inflammatory, anti-oxidant and anti-proliferative contents so as to possibly cure or prevent psoriasis by S. oleracea and improve quality of life of psoriatic patients in a cost-effective manner.

MATERIALS AND METHODS

Preparation of spinach extract
The whole plant of Spinacia oleracea was collected and submitted for identification to Herbarium, University of Karachi. The plant after identification was issued specimen identification number G.H. No: 93373. Fresh leaves of S. oleracea were washed; cut into small pieces and macerated overnight at a proportion of 50gm in 200ml distilled water. Leaves were then well-triturated in the macerated solvent using mortar and pestle. The final volume of the extract was made up to 300ml by distilled water to make it convenient to administer.

Dose calculations
National Cancer Institute (NCI), USA recommends a daily intake of 5-6 mg beta-carotene while The German Nutrition Society-Deutsche Gesellschaft fur Ernahrung (DGE) recommends daily 2 mg of beta-carotene along with retinol equivalents for vitamin A. However 100gm spinach is reported to provide 17.3 mg carotenoids (Muller 1996).

In view of NCI guidelines to reduce psoriatic patches, if 34 gm S. oleracea is given it will provide 6 mg of carotenoid, hence for an average weight 0.18 kg albino rat, 0.08 gm S. oleracea will provide the daily recommended 0.01 mg carotenoids. In this study, the extract of S. oleracea was prepared in the concentration of 50 gm in 300 ml of distilled water, thus 0.08 gm S. oleracea 0.5 ml S. oleracea extract.

However in view of DGE guidelines to reduce PASI score, if 11.5 gm S. oleracea is given it will provide 2 mg of carotenoid, hence for an average weight 0.18 kg albino rat, 0.016 gm S. oleracea will provide the daily recommended 0.0005mg carotenoid, thus 0.016 gm S. oleracea 0.1 ml S. oleracea extract. Hence, 0.5ml was considered as preventive dose of S. oleracea for percentage reduction of the psoriatic patch and 0.1 ml was considered as preventive dose in PASI while their respective doubles i.e. 1ml and 0.2ml were hypothetically set as therapeutic (curative) doses of S. oleracea extract.

Calibration of Vernier Caliper
Vernier caliper was checked for the presence of zero error at the start of the study. It was calibrated weekly by placing the standard length of 25mm between the jaws of Vernier caliper at three different positions to determine the length and observations were noted. The calibration report was satisfactory. Tolerance limit of Vernier caliper was ± 0.05mm or 0.005 cm.

Preparation of solutions
Methotrexate solution was prepared freshly before administration in a concentration of 2mg/10ml, which was given orally on a weekly basis. The folic acid solution was made in a concentration of 5mg/10ml and given daily except on the day of methotrexate administration. Potassium iodide solution prepared by dissolving 1g potassium iodide in 100ml distilled water through continuous stirring with the help of glass rod until a clear solution was obtained. The solution was then stored in tightly capped amber-colored glass bottle for further use.

Induction of Psoriasis
Psoriasis was induced in all rats by 1% potassium iodide solution, 0.1 ml of this solution was given once a day by the oral administration until psoriasis was induced (Shelley 1967; Hassan and Keen 2012).

Animals
Forty albino rats of either gender weighing 180-200 gm were procured from Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi and retained in plastic cages in the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences under strict conditions of temperature (22 ± 2˚C) and humidity (50-60%). The study was conducted after the approval from Board of Advanced Study and Research (BASR), University of Karachi. Forty rats were separated equally into two groups then each group was further sub-divided into four groups designated as standard, preventive, therapeutic and control with five rats in each group.

Procedure
Percentage reduction in psoriatic patch size determined Anti-psoriatic activity of S. oleracea according to National Cancer Institute, USA guidelines and psoriasis area and severity index (PASI) score according to DGE guidelines with slight modification i.e. preventive doses were according to guidelines, but curative doses were double to that of preventive treatments, while rest of the procedure in both the methods was same.

After induction of psoriasis, a test dose of 0.05ml methotrexate was given orally to animals of the standard group to observe adverse reaction if any. Then 0.2ml of methotrexate was given once weekly and 0.02ml of folic acid was given once daily to this group except for the day
of methotrexate dose (Menter et al. 2008). The animals in control group were only given 1ml distilled water orally throughout the study after induction of psoriasis to assess the role of immune system in the healing of psoriatic patches.

**Therapeutic Group (Curative)**

I. **Percentage reduction in psoriatic patches**

Animals in the curative group were given 0.16gm (1ml) of *S. oleracea* extract orally once a day from day 11 up to the end of the study and percentage reduction in the size of psoriatic patches was determined.

II. **Psoriasis area and severity index score (PASI)**

Animals in this procedure were given 0.06gm (0.2ml) of *S. oleracea* extract orally per day from day 11 up to the end of the study and PASI score was determined.

**Preventive Group**

I. **Percentage reduction in psoriatic patches**

Albino rats in this group were simultaneously given 0.1ml potassium iodide solution and 0.08gm (0.5ml) of *S. oleracea* extract orally per day up to the end of the study and percentage reduction in the size of psoriatic patches was determined.

II. **Psoriasis area and severity index score (PASI)**

Albino rats in this group were simultaneously given 0.1ml potassium iodide solution and 0.03gm (0.1ml) of *S. oleracea* extract per day up to the end of study and PASI score was assessed.

**Evaluation of Psoriatic patches**

1. **Percentage reduction of psoriatic patch surface area**

Psoriatic patch size was measured with periodic interval of up to 120 days using Vernier caliper and scale. Reduction in psoriatic patch size was determined using the formula with certain modification as suggested by South West Regional Wound Care Toolkit in 2011.

   \[
   \text{Percentage reduction in psoriatic patch size} = \frac{\text{SAI} - \text{SAF}}{\text{SAI}} \times 100
   \]

   Where,

   - SAI = Initial surface area of psoriatic patch (after induction of Psoriasis)
   - SAF = Final surface area of psoriatic patch (after methotrexate or *S. oleracea* doses)

   The delay in development of psoriatic patch in the preventive group was also noted as it shows the effectiveness of *S. oleracea* in slowing down flare-up process of psoriasis in comparison to the control group.

   Statistical analysis was used to draw the outcomes and significance of the study by applying t-test between standard and curative, preventive and control groups of albino rats.

2. **PASI score irrespective of affected skin area**

PASI score was assessed using three symptoms, erythema (redness), induration (thickness of psoriatic patch) and desquamation (scaling) without considering the overall affected area of skin. The skin of rats is partially shaved.

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**Fig. 1**: shows normal albino rats before induction of psoriasis

**Fig. 2**: Control rat with psoriatic patches

**Fig. 3**: Reduction in psoriatic patch size *p<0.05 significant as compared to control group

**Fig. 4**: showing standard deviations in each group of albino rats with error bars
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Fig 5: Rats received methotrexate after psoriasis

Fig 6: Rats received S. oleracea in preventive doses

to remove furs so as to properly visualize the redness, thickness and scaling of the skin. The score was taken weekly from 0 to 4 by severity of the symptoms as mentioned above. 0 indicates the absence of symptoms, 1 for mild, 2 for moderate, three for severe and four as very severe (Van Der Fits et al 2009; Coimbra 2012). Statistical analysis was applied to the noted observations to draw the significance.

RESULTS

Percentage Reduction of Psoriatic patch
Figure 1 shows normal albino rats at the start of the study before induction.

Figure 2 shows the psoriatic patches in control animal after induction. Size of psoriatic patch in this group is comparatively large since healing of the psoriatic patch depended on the immune system.

Figure 3 and table 1 show comparison in the reduction of the psoriatic patch size at the curative dose of S. oleracea and 0.2ml methotrexate solution in rats.

There was a significant reduction in patch size after day 11 in both groups. However, reduction in the psoriatic patch size after day 20 at preventive doses of S. oleracea was also significant compared to the control group of rats.

Table 1: Reduction in psoriatic patch size by S. oleracea at curative dose

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>SAI (cm)</th>
<th>SAF (cm)</th>
<th>% reduction in psoriatic patch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>9.5</td>
<td>4.4</td>
<td>53.68</td>
</tr>
<tr>
<td>S. oleracea Curative dose</td>
<td>9.5</td>
<td>5.6</td>
<td>41.05</td>
</tr>
</tbody>
</table>

After 120 days, percentage reduction of psoriatic patches was 53.68% and 41.05% by methotrexate and the curative dose of S. oleracea respectively. The psoriatic patch size was 9.6 cm at Day 11 in the control group, however, in rats received S. oleracea in preventive dose the same size psoriatic patch was developed in 20 days.

Significance was determined by t-test. The p-values less than 0.05 were considered significant and values less than 0.01 were considered highly significant. Figure 4 shows standard deviations in each group of albino rats with error bars.
Figure 5 shows rats that were given methotrexate after induction, the size of psoriatic patch is comparatively small, showing the faster healing of psoriatic patch by standard drug.

Figure 6 shows albino rats received *S. oleracea* in preventive doses, size of psoriatic patch in these animals is smaller than that of control rats.

Figure 7: Albino rats received *S. oleracea* in curative doses.

**PASI score irrespective of affected skin area**
Figure 8 shows albino rats while assessing erythema, induration and desquamation parameters of PASI score.

Redness and scaling was prominent while more thickened skin than normal can also be seen due to psoriasis.

Figure 8: Albino rats showing affected skin pattern in PASI score.

Figure 9 shows decrease in erythema by methotrexate and *S. oleracea* at curative and preventive doses as measured by PASI score. The decrease in redness (erythema) of psoriatic patches was significant with methotrexate and curative dose of *S. oleracea*, however, decrease in redness was highly significant at the preventive dose as compared to control group.

Figure 9: Albino rats showing decreased erythema by methotrexate and *S. oleracea*.

Figure 10 shows a comparison of the decrease in induration by methotrexate and *S. oleracea* at curative and preventive doses as measured by PASI score. The reduction in thickness (induration) of psoriatic patches was highly significant by methotrexate and the curative dose of *S. oleracea*, however, decrease in induration was substantial at preventive dose of *S. oleracea* as compared to control group.

Figure 10: Albino rats showing decreased induration by methotrexate and *S. oleracea*.

Figure 11 shows the decrease in desquamation of psoriatic patches by methotrexate and *S. oleracea* at curative and preventive doses as measured by PASI score. There was highly significant reduction in desquamation by methotrexate and curative as well as preventive doses of *S. oleracea* as compared to control group.

Figure 11: Albino rats showing decreased desquamation by methotrexate and *S. oleracea*. 
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Statistical analysis was performed by applying t-test to PASI scores and p-values were obtained which showed decrease in intensity of psoriasis as observed by decrease in erythema, induration, and desquamation at curative and preventive doses of *S. oleracea*.

**DISCUSSION**

Psoriasis is a chronic, non-contagious, painful inflammatory disorder that disturbs the quality of life and is considered as a topic of global concern by WHO due to...
its upward trend of prevalence worldwide. In continued endeavors to prevent and cure psoriasis, various public awareness programs have been arranged by WHO, CDC, and EADV. Its treatment is mainly symptomatic and to date, no therapy has found to be beneficial in complete cure of this disease as well as eliminating the risk of relapse.

Hence this study was aimed to evaluate anti-psoriatic effects of aqueous extract of *S. oleracea* in rats in preventive and curative doses. There was 53.68% reduction in psoriatic patch surface area by standard drug methotrexate after 120 days, however reduction by the curative dose of *S. oleracea* was 41.05%, thus revealing that *S. oleracea* was effective in the healing of psoriatic patch. This may be due to the presence of antioxidant and anti-inflammatory carotenoids in *S. oleracea* (Menter et al 2008; Van Der Fits et al 2009; South West Regional Wound Care Toolkit 2011). The size of the psoriatic patch in control group was 9.6 cm at day 11, however in animals received preventive doses of *S. oleracea* it took 20 days to attain 9.6 cm size of the psoriatic patch, hence there was a delay of 9 days in the progression of the psoriatic patch which shows protective effects of spinach against psoriasis. The results were significant for animals received methotrexate, curative and preventive doses of *S. oleracea* as compared to the control group of rats.

The intensity of psoriasis as measured by a decrease in erythema, induration, and desquamation as described by PASI criteria that is 0 absence, 1 mild, 2 moderate, 3 severe and 4 very severe. After 16 weeks, animals received methotrexate and *S. oleracea* in curative doses showed a significant reduction in erythema and highly significant reduction in induration and desquamation. Animals received *S. oleracea* in preventive doses showed a highly substantial decrease in erythema and desquamation and a significant reduction in induration as compared to the control group of rats. The reason for decrease in severity and patch size was may be due to presence of carotenoids in *S. oleracea* (beta-carotene, lutein, zeaxanthin, astaxanthin, neoxanthin, and violaxanthin) along with flavonoids (spinacetin, patuletin, jaceidin) and other phytochemicals since there are reports which suggest that *S. oleracea* possesses anti-inflammatory, anti-oxidant, anti-proliferative and skin-strengthening contents e.g. carotenoids, flavonoids and other phytochemicals. It also contains vitamin A, vitamin B complex, vitamin C, folate and trace elements that combat various skin and other abnormalities (Julie and Elizabeth 2010; Lima and Kimbal 2011; Machado 2017). Hence *S. oleracea* can be taken as a cost-effective preventive and curative therapy for psoriasis.

**CONCLUSION**

It can be concluded that *S. oleracea* possesses curative and preventive effects on psoriatic patches as determined by percentage reduction in surface area of the psoriatic patches according to NCI, USA guidelines and PASI score according to DGE guidelines. Hence *S. oleracea* may be used in the prevention and treatment of the psoriasis without any risk of adverse effects.

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