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Effect on ethanolic extract of *Sechium edule* fruitson imquimod-induced psoriasis like dermatitis in wistar rats

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Abstract: The purpose of this study was to find out if the ethanolic fruit extract of *Sechium edule* fruits could prevent Imquimod (IMQ)-induced psoriasis-like dermatitis in male Wistar rats. The rats were divided into four groups of five rats each group. Group 1 served as a negative control, while groups 2 and 4 received 5 percent IMQ cream topically on shaved backs, topical 5 percent IMQ cream + *S. edule* (200mg/kg) orally once daily and topical 5 percent IMQ cream + *S. edule* (400mg/kg) orally once daily, respectively. From days 3 to 9, the animals treated with IMQ developed characteristic erythmea, scaling and thickening, according to the findings. Furthermore, skin thickness and the psoriasis area severity index (PASI) both were increased significantly. In IMQ-challenged mice, histological investigation revealed epidermal cuticle, including parakeratosis, acanthosis and perivascular infiltration of inflammatory cells. In IMQ-challenged rats, treatment with *S. edule* (200 and 400mg/kg) significantly reversed all of these symptoms.

Keywords: *Sechium edule,* Imiquimod, psoriasis, dermatitis and parakeratosis.

INTRODUCTION

Psoriasis is a widespread chronic inflammatory skin disease that affects 1% to 3% of the global population. It is characterised by localised or generalised skin lesions, such as erythematous plaque and lamellar scales (Boehncke and Schon, 2015). Environmental, obesity, genetic, stress and immunologic variables all appear to contribute to the progression of this disease (Ottawa, 2009). Elbows, knees and the scalp are the most usually affected areas. Scaling, itching, erythema, burning and bleeding are all symptoms of psoriasis. Plaque, pustular, inverted, napkin and guttae are the most common kinds of psoriasis (Palfreeman*et al.,* 2013). Psoriasis histological abnormalities include epidermal keratinocyte hyperproliferation and poor differentiation, increased skin vascularization, and leukocyte infiltration, which include T cells, macrophages, dendritic cells and neutrophils (Meng *et al.,* 2017). In psoriasis, it acts as a primary pathogenic actor by activating T cells and producing cytokines and chemokines. Inflammatory infiltrates and the development of psoriasis may be caused by dendritic cells in the dermis (Nestle *et al.,* 1994). Dendritic cells are the most significant professional antigen-presenting cells in the body, migrating towards immunological organs to offer processed antigens to T cells and trigger the specific immune response (Krueger and Bowcock, 2005; Nestle *et al.,* 2009; Luo *et al.,* 2016).

Imquimod (IMQ)-induced psoriasis-like inflammations are mediated by the IL-23/IL-17A axis, which leads to fast dendritic cell proliferation and keratinocyte activation, resulting in increased cytokine production. CD4+ T-helper cells Th1 and Th17 infiltrate the dermis and release pro-inflammatory cytokines interferon-γ, TNF-α and IL17A, IL-17F and IL-22 (Meng *et al.,* 2017; Di *et al.,* 2016; Dimitris *et al.,* 2020). The first line of treatment for psoriasis has been established standard systemic medicines such as methotrexate, cyclosporine and acitretin (Palfreeman*et al.,* 2013). These agents, on the other hand, appear to have a slew of major side effects. As a result, alternative medicine with lower toxicity is required.

Sechium edule, commonly known as chayote, choko, chocho, chow-chow and vegetable pear, is an edible plant that belongs to the curcurbitaceae family. This plant contained eight flavonoids, including three C-glycosyl and five O-glycosyl flavones (Siciliano and De 2004). The leaves and fruits possess increase in urine output, cardiac protection, as well as reduction of oedema. In addition, leaves are used to treat atherosclerosis, hypertension and kidney stones (Vieira *et al.,* 2019). Liver protection (Firdous *et al.,* 2012), ulcer reduction (Firdous

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et al., 2012), decrease in convulsion and central nervous system depression (Sayeed *et al.,* 2012), kidney protection (Sayeed *et al.,* 2013), blood glucose lowering Maity*et al.,* 2013), and antioxidant properties have all been documented for the fruits of *S. edule* (Ordonez *et al.,* 2006). There has been no activity on *S. edule* psoriasis reported till today. As a result, the current study was designed to assess the antipsoriatic effect of *S. edule* fruits in rats with IMQ-induced psoriasis.

MATERIALS AND METHODS

Drugs and reagents

IMQ Cream (Glenmark pharmaceutical Pvt. Ltd.), Ketamine HCl (Vulcan laboratories Pvt. Ltd, Kolkata and India), Saline water (Baxter Pvt. Ltd.) were used for the study. Other reagents used in the study were analytical grade.

Materials

Vernier caliper scale (Esel international Pvt. Ltd.), Trimmer (Philips Pvt Ltd.) and light microscope (ESAW, India), slides and cover slips.

Plant materials

Fruits of *S. edule* were acquired from a local market in Kolkata. The fruits were systematically distinguished and confirmed at Regional Research Institute, Bangalore (RRCBI/MCW/7/2008).

Extraction

S. edule fruits were cleaned with fresh water and then with double distilled water. Fruits were then chopped into small pieces and kept for drying. Mechanical grinding was done to obtain the powdered *S. edule* fruit. Dried powder was defatted using petroleum ether (bp 60-80^{o}C) for 72 hours and then maceration was conducted using ethanol for 72 hours with intermittent shaking for ethanolic extract preparation. Filtration was done and then distillation was performed to remove the solvent. The product hence obtained was reduced to a dark colored mass by keeping in boiling water bath for further solvent elimination. This part of the sample was the ethanolic extract. The extract was refrigerated for storage (Firdous *et al.,* 2012).

Acute Oral Toxicity Study

Male Wistar rats were fasted overnight before receiving the drugs. After that, a single oral dose of *S. edule* fruits ethanolic extract (2000mg/kg) was given. For 3-4 hours, the animals were kept under observation and their diets were withheld. They were observed twice after the first 30 minutes of dosing and then daily for the following 14 days (OECD 2002). They were watched two times after the first 30 minutes of dosing and then every day for the next 24 hours (with particular notice during the initial 4 hours are much necessary).

Laboratoryanimals

Male Wistar rats about 180-220g in weight were utilized in the research. The rodents were kept up under controlled states of environment (23±2°C) and dampness (52±2%). They were placed in sterilized cages made up of polypropylene. Sterile paddy husk was used for bedding. Their growth was maintained under standard rodent pellet to ensure rodents libitum weight. It took a week for the rats to get adapted with the laboratory conditions. All the test techniques were performed by committee for the purpose of control and supervision of experiments on animals (CPCSEA), service of social equity and strengthening Government of India, standards and affirmed by the Institutional Animal Ethics Committee (IAEC) (Ref. No. F4/CIPT/ADMIN/2020-21/006)

Experimental design and S. edule extract administration

The animals were split into 4 groups, each with five animals. A trimmer was used to remove hair from the rats' backs. The first group was used as a negative control group, receiving just saline water and rat pellet diet. The second group was given a daily topical dose of 62.50mg of 5% IMQ cream on their shaved backs as a positive control. The third group received topical 62.50mg of 5% IMQ cream + *S. edule* (200mg/kg) once daily, whereas the fourth group received oral gavages of topical 62.50mg of 5% IMQ cream + *S. edule* (400mg/kg) once daily. The treatment lasted nine days. The animals were then anesthetized with ketamine (50mg/kg). Central dorsal skin tissues (approximately 1cm^2) from all the groups were excised for histological studies (Sun *et al.,* 2013; Luo *et al.,* 2016; Di *et al.,* 2016; Dimitris *et al.,* 2020).

Scoring severity of skin

The degree of erythema, thickness and scaling on the affected dorsal skin surface was evaluated to determine psoriasis area and severity index (PASI) scores. On a four-point scale, PASI was calculated for each (0 = none; 1 = slight; 2 = moderate; 3 = marked; 4 = very marked). The total scores (erythema plus scaling plus thickness) were used to determine the degree of skin inflammation, which ranged from 0 to 12. Every other day, the thickness of the skin was measured with digital callipers (Sun *et al.,* 2013).

Histological Studies

For the histopathological study skin tissues were treated for formalin fixation. After an overnight formalin fixation, the moisture level of the rat specimens were reduced by washing with alcohol and benzene and then fixed with paraffin wax. About 5 µm thick blocks were made by double stain technique using eosin and hematoxylin. The blocks were observed under light microscope (Firdous *et al.,* 2012).

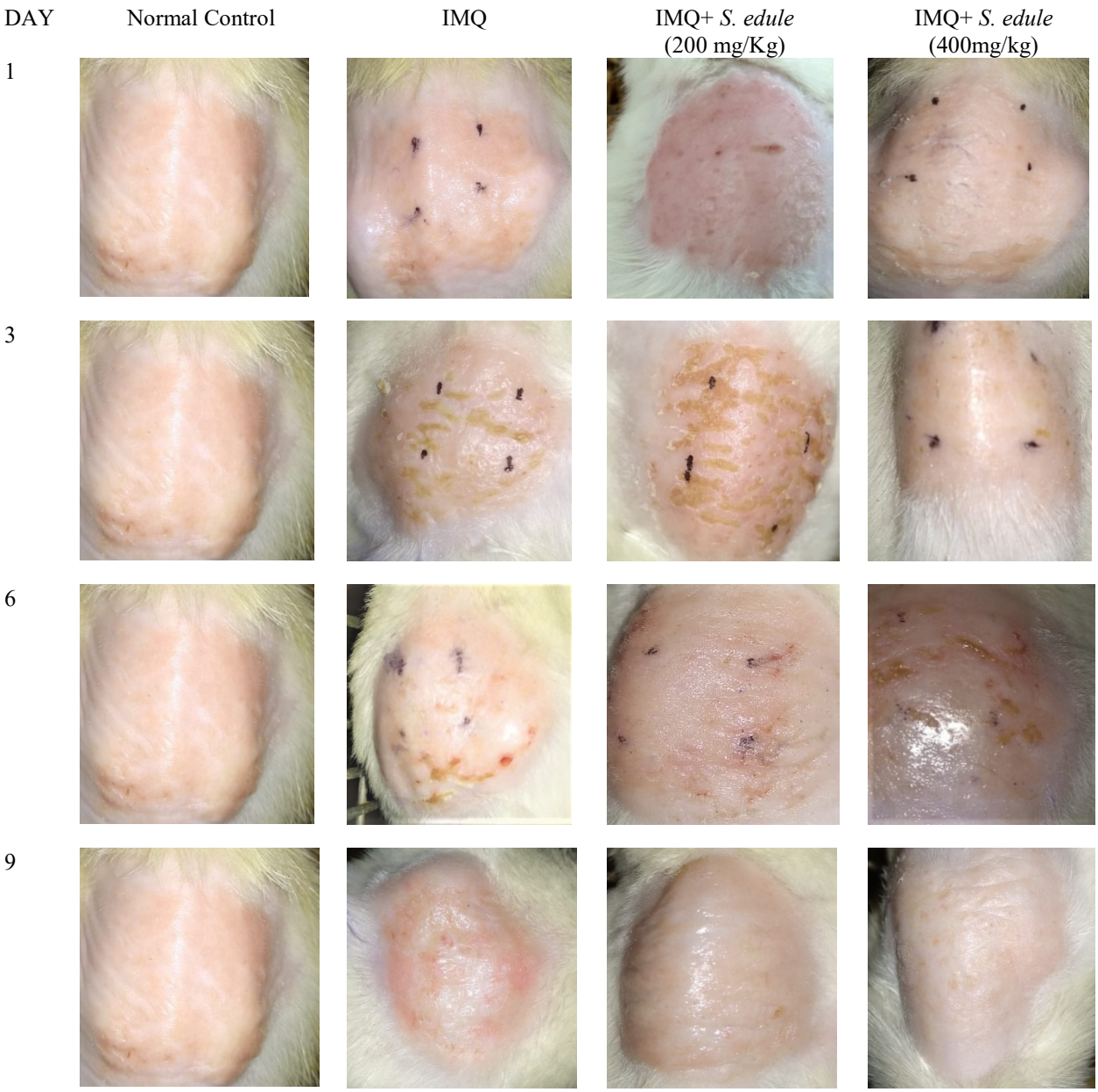

Fig. 1: Comparison of the morphological observations of back skin in the different groups after IMQ exposure for nine days

Table 1: Effect of ethanolic extract of *S. edule* on skin thickness of psoriatic rats

Treatment	Days								
	01	02	03	04	05	06	07	08	09
Normal Control	2.02± 0.0086	2.038± 0.006	2.022± 0.0086	2.04± 0.0070	2.03± 0.0070	2.04± 0.013	2.04± 0.0070	2.05± 0.017	2.05± 0.010
IMQ	2.28± 0.27$^{###}$	2.45± 0.056$^{###}$	2.61± 0.030$^{###}$	2.57± 0.0070$^{###}$	2.65± 0.029$^{###}$	2.65± 0.026$^{###}$	2.70± 0.031$^{###}$	2.69± 0.03$^{###}$	2.74± 0.034$^{###}$
IMQ+*S. edule* (200mg/kg)	2.192± 0.020*	2.23± 0.015***	2.304± 0.011***	2.30± 0.0087***	2.35± 0.012***	2.37± 0.010***	2.46± 0.022***	2.42± 0.010***	2.50± 0.013***
IMQ+*S. edule* (400mg/kg)	2.042± 0.010***	2.046± 0.011***	2.06± 0.010***	2.08± 0.016***	2.09± 0.010***	2.13± 0.017***	2.16± 0.014***	2.26± 0.020***	2.28± 0.021***

Data are presented as Mean±SEM. $^{###}$P<0.001 when compared with Normal Control group. *P<0.05 and ***P<0.001 when

Histological examination of the rat back skin in different groups

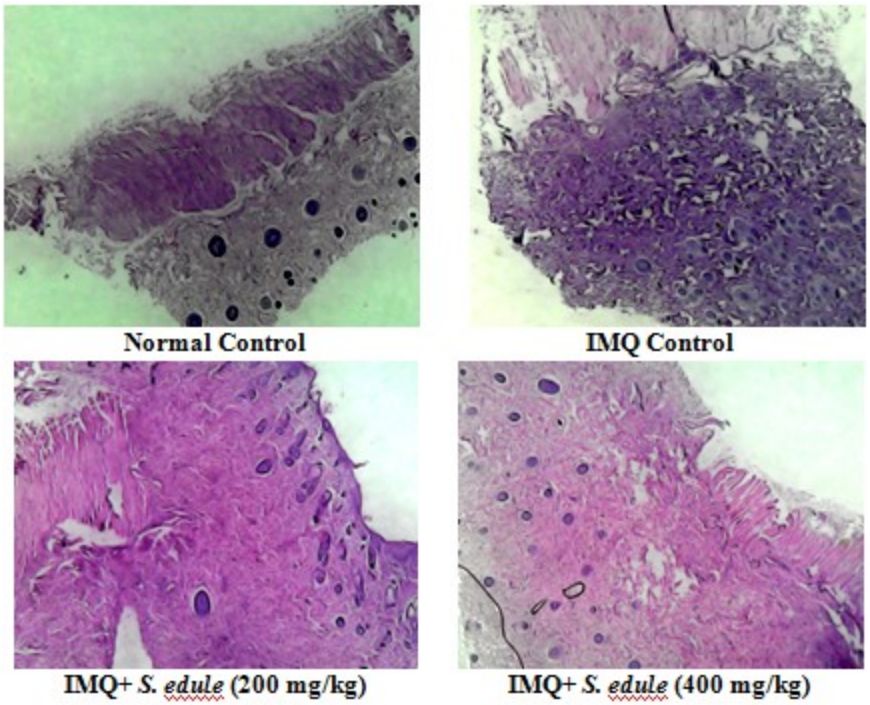

Fig. 2: Histological analyses of rat back skin in different groups (Magnification: x100)

Table 2: Average score of PASI of different groups

Groups	Days								
	01	02	03	04	05	06	07	08	09
Normal Control	0	0	0	0	0	0	0	0	0
IMQ	0	1.6	1.8	2.6	2.8	3.2	4	4	4
IMQ + *S. edule* (200 mg/kg)	0	1.6	2	2	2	3	2	2	2
IMQ + *S. edule*(400 mg/kg)	0	1	1	2	2	2	1	1	1

Data are represented as mean.

RESULTS

Morphological observations of back skin of different groups after IMQ exposure

Fig.1 below showed typical erythmea, scaling and thickening of the back skin of IMQ challenged animals (second group) on day 3, 6 and 9 as compared to the normal control group (first group). Recovery was observed in rats fed with 200 and 400 mg/kg of *S. edule* fruit extract (third and forth group, respectively) on the ninth day (day 9) compared to IMQ group (second group). Results of the effect of *S. edule* extract on skin thickness in IMQ-induced psoriatic rats are showed in table 1. Animals (second group) showed a notable rise in skin thickness from day 1-9 when compared with normal control animals. IMQ challenged animals showed gradual increase in skin thickness and maximum thickness was observed on day 9. Animals treated with *S. edule* (200 and 400mg/kg) showed a considerable decline in skin thickness from day 1 to 9 in comparison with IMQ control animals (second group). However, more prominent protective action was observed from day 6 to day 9.

Further, PASI score was evaluated in different group of animals from day 1-9 (table 2 and fig. 1). We found that there was a notable increase in PASI score in IMQ control animals (second group). Nevertheless, Animals treated with *S. edule* (200 and 400mg/kg) showed a considerable decline in PASI score from day 1 to 9.

From fig. 2 we came to know that control group shows normal appearance of the general tissue structure of the skin. Skin treated with IMQ demonstrated pathological changes of the epidermal cuticle, including parakeratosis, acanthosis and perivascular infiltration of inflammatory cells in the upper dermis. Animals treated with extract

(200 and 400mg/kg) showed improved architecture of the skin tissue. However, the higher dose of the extract showed more prominent effect. Hence, histological analysis of the skin tissue revealed marked improvement of the tissue architecture by *S. edule* fruit extract.

STATISTICAL ANALYSIS

All results are presented as mean ±standard error of mean (SEM). One way analysis of variance (ANOVA) was utilized in comparing the difference within groups, followed by Tukey's Multiple Comparisons Test using Graph pad Prism version 8.1. The level of significance was placed at $P<0.05$.

DISCUSSION

Inflammatory cytokine release, immune cell infiltration into the skin and hyperkeratosis characterize the skin reaction in psoriasis (Rendon and Schäkel, 2019; Wollina*et al*., 2020). IMQ-induced psoriasis-like dermatitis is because of resulting in increased cytokine production. CD4+T-helper cells (mainly Th1 and Th17) infiltrate the dermis and release several pro-inflammatory cytokines like interferon-γ, TNF-α and a number of interleukins involved in inflammation (Meng *et al.,* 2017; Dimitris *et al.,* 2020).

In our study on different days, IMQ-challenged animals showed normal erythmea, scaling and thickening of the back skin, while rats fed with *S. edule* extract showed a noteworthy recovery. We found that the degree of erythema, thickness and scaling on the affected dorsal skin surface was notably increased in psoriasis control animals as evidenced by decrease in PASI scores. From day 1 to day 9, the extract exhibited a considerable reduction in skin thickness and PASI score. However, from day 6 to day 9, the extract appeared to have a stronger protective effect. Furthermore, the extract reduced histological abnormalities caused by IMQ application, such as epidermal cuticle, parakeratosis, acanthosis and perivascular infiltration of inflammatory cells.

The utilization of plants, natural herbs and spices has made a significant contribution to today's disease-fighting strategy. Flavonoids, a phytochemical found in plants, have recently been discovered to have potent anti-inflammatory actions (Maleki*et al.,* 2019). Paeonol, a natural chemical derived from *Paeonia suffruticosa*, was found to diminish imiquimod-induced psoriasis in mice by inhibiting dendritic cells, according to Meng *et al.,* (2017). In the *S. edule* plant, eight flavonoids were discovered, including three C-glycosyl flavones and five O-glycosyl flavones (Siciliano and De 2004). As a result, the presence of these flavonoids may be responsible for protective effect of *S. edule* against IMQ-induced psoriasis/dermal inflammation. This finding suggests that *S. edule* could be effective as a therapeutic alternative for psoriasis treatment.

CONCLUSION

S. edule extract provides a preventive effect in a rat model of IMQ-induced psoriasis, according to our findings. When compared to the IMQ-treated mice, the extract shows a significant reduction in skin thickness and improves PASI score. The extract also enhances the skin's histological characteristics. This research implies that *S. edule* could be a viable therapeutic option for the treatment of psoriasis.

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