Panacea Journal of Medical Sciences 2020;10(2):108–113

Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: www.ipinnovative.com

Original Research Article

Efficacy of topical soy Isoflavonoid extract in prevention and reversal of photo-ageing in guinea pig model

T Padmavathi[1], I Israel Raja Johnley[1,*], Prashanth Jeyabaskaran[2]

[1]*Dept. of Pharmacology, Government Thoothukudi Medical College, Thoothukudi, Tamil Nadu, India*
[2]*Government Thoothukudi Medical College, Thoothukudi, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 16-04-2020
Accepted 01-07-2020
Available online 26-08-2020

Keywords:
Photoaging
UVB radiation
Isoflavonoid
Erythema scale
Guinea pig
Tretinoin

ABSTRACT

Background: Photo aging is the superimposition of photodamage on intrinsically aged skin generally bringing about premature aging due to chronic and multiple exposure of the skin to UV light. Routinely used retinoids have many disadvantages. Phyto-estrogens present in plants have gained recent popularity as anti-photo aging compounds. Isoflavonoids such as soy compounds can provide good photoprotection of human skin. We intended to study efficacy of topical preparation of isoflavones in guineapigs for treatment and prophylaxis of photoaging.

Materials and Methods: Three groups of guinea pigs were formed with 3 in each. Group II was pretreated with isoflavonoid. All the groups received irradiation with UV-B for 8 weeks. After irradiation Group I was treated with isoflavonoid and group II with tretinoin for 4 weeks. Erythema was scored with erythema scale before and treatment.

Results: Group II pre treated with isoflavonoid developed erythema only after 4 weeks where as other two groups developed in first week of irradiation. After treatment with isoflavonoids for 4 weeks, Group I guinea pigs recorded complete reduction in erythema from 3to 0. Group III which were treated with tretinoin recorded decline in erythema from 3 to 0.6. Guinea pigs of Group II had erythema score of 1 which persisted up to 8th week.

Conclusion: Isoflavonoid very well prevented erythema in guinea pig skin after UV-B irradiation. Topical isoflavonoid nullified erythema fully in phototoxicity. Efficacy of isoflavonoid is comparable to that of tretinoin.

1. Introduction

Aging is a process continuum that results due to a genetic program or a clock that is implanted in the genetic make-up of each species. Skin aging has been found to be caused by several factors such as genetic condition, environmental exposure (UV radiation, xenobiotics, and mechanical stress), hormonal changes and metabolic processes. All the fore-mentioned factors together act on the alterations of skin structure, function, and appearance. Yet it has been reported worldwide that solar UV radiation unquestionably is the single major factor responsible for skin aging.

Intrinsic or chronological aging is defined by the clinical, histological, and physiological decrements that occur in the sun-protected skin, affecting the rate of epidermal turnover, clearance of chemical substances from the dermis, dermal thickness and cellularity, thermoregulation, rate of re-epithelialization after wounding, immune responsiveness, sensory perception, sweat and sebum production, capacity for vitamin D synthesis and vascular reactivity. Clinically, the intrinsically aged skin is atrophic, which may result in prominence of vasculature and loss of elasticity.

Photoaging is the superimposition of photodamage on intrinsically aged skin generally bringing about premature aging. This specific damage occurs by chronic and multiple exposure of the skin to UV light. Clinically, the skin

* *Corresponding author*.
E-mail address: irjzion@gmail.com (I. I. R. Johnley).

https://doi.org/10.18231/j.pjms.2020.025

becomes coarse due to thickening of epidermis initially and thinning of dermis later on. Ultimately, in photoaging there is laxity, sallowness with wrinkles, irregular hyperpigmentation, lentigines, and telangiectasias.[1] Although both photoaging and chronological aging are associated with wrinkles, photoaging-induced wrinkles are regarded as deeper and more coarse, whereas those associated with chronological aging are generally more superficial and delicate.[2] Hence photoaging that occurs due to sunlight throws more concerns among medical professionals and it is highly necessary to prevent UV induced skin damage rather than treating this complication.

Of many compounds being used for therapy and prevention of aging process of skin, retinoids are very well known to influence a variety of cellular processes, such as cellular growth and differentiation, cell surface alterations, and immune modulation. Many of their tissue effects are mediated by their interaction with specific cellular and nucleic acid receptors. Tretinoin happens to be the retinoid that is investigated more than any other retinoid implicated in the treatment of intrinsic or photoaging with more success. Even though the conventional tretinoin therapy has proved to yield good results it is not without disadvantages which can be enumerated as slower rate of beneficial effects over a longer period of time and prominent adverse effects like irritation, erythema and dermatitis.[1] Hence we need to seek for alternate compounds which pave way for early recovery with minimal or tolerable side effects.

There is an ever-increasing interest in anti-aging substances derived from food. Since the aging process inevitably involves the generation of reactive oxygen species, oral supplements with antioxidant properties are the most popular. These include botanicals with carotenoids or polyphenols, isoflavones, vitamins, coenzyme Q10, phytoestrogens, probiotics and omega-3 fatty acids.[3]

Phyto-estrogens (plant estrogens) are non-steroidal, naturally occurring compounds that exert estrogenic effects in all aspects including skin of our body. They are structurally similar to naturally occurring estrogens. Phyto-estrogens have many classes, the most common being isoflavones, lignans and coumestans. Isoflavonoids are a class of flavonoids having the (3 - phenyl - 1, 4-benzopyrone) structure. Flavonoids are dietary factors that are synthesized and seen as pigments and secondary metabolites in plants mainly in leguminous plants. In higher plants flavonoids are used in UV filtration. Isoflavones are present in the highest amount in soybeans, flaxseed, and legumes with genistein, daidzein, and glycitein being the most common type. Soy is a staple of Asian diets with daily intake at least 40 times higher than that observed among western populations. They are not present in large quantities as biologically active forms in naturally occurring substances instead they are found as precursors.

Isoflavonoids which act on the oestrogen receptor may either have a stimulating or an inhibitory effect on the estrogen receptor (ER). Of the two types of estrogen receptors such as ER alpha and ER beta, it is found that phytoestrogens have higher affinity towards ER beta.[4] Even though studies performed on skin cells in vitro or on animal models suggest that oral uptake of selected micronutrients and phytochemicals can provide photoprotection of human skin, this can only be achieved if an optimal pharmacological dose range is reached in the human skin. These phyto estrogens are inactive molecules in glyosidic form. They become active as aglycones only after intestinal absorption and subjected to first pass metabolism. They have low bioavailability when given orally. This can be bypassed by topically applying them on localized areas. As topically applied isoflavonoids are not subjected to first pass metabolism, better pharmacological concentration in skin can be achieved.

Among the various animals being used for animal studies, mice are commonly used as skin disease models. C57BL/6J, SKH1, and BALB/c mice are the three main strains used for photo aging and photo carcinogenesis studies.[2] But many inherent differences have been found in skin structure between mice and human skin. The disadvantage of using mice as a model is that the thicknesses of mouse skin and human skin are not exactly the same, nor are the human and murine responses to solar simulated radiation.[2] As guinea pigs possess many biological similarities to humans, beyond the simple fact that they are mammals, they have been used as experimental animals for centuries in many fields of research.[5] Unlike guinea pigs, mice do not develop marked erythema and therefore edema development is used for evaluation.Since human skin and guinea pig skin are found to be very similar in architecture, this model may reflect what would occur in human skin. In order to develop compounds that may protect against sun damage, hairless mice and guinea pigs have mainly been used.[6] Considering all these facts, we have decided to use guinea pigs for inducing a photo aging model in the present study.

The aim of this research is to acquire more comprehensive understanding of the iso flavonoids mediated photo protection in guinea pig skin model which resembles human model. This study has been undertaken also to analyze preventive and reversal inducing properties of iso flavonoids against photo-ageing in guinea pigs.

2. Materials and Methods

Our study was conducted in the Central Animal house, Government Thoothukudi Medical College between September 2019 to December 2019, in accordance with guidance from the Institutional Animal Research Ethics Committee after obtaining ethical approval.

Guinea Pig Surface Model was used for testing phototoxicity using UV-B light. A total of 9 guinea pigs were selected from the animal house and used for this study. Three groups of animals were formed with 3 guinea pigs to each group. Animals of Group I were used for the treatment with iso flavonoids, Group II animals were pre-induced with isoflavonoids and guinea pigs of group III received standard treatment with tretinoin. All the animals were acclimatized 15 days prior to study and were given free access to food and water. About 48 hours prior to the treatment, hair was removed from an area of size 3 x 4 cm on the back with a fine clipper.

All the guinea pigs assigned to Group I and III were exposed to UV-B irradiation (Figure 1).

Fig. 1: UVB radiation

During induction, the heads were covered to prevent eye damage. A paste formed by mixing ethanolic extract of isoflavonoids with petroleum jelly was uniformly applied over the prepared area for all the guinea pigs of group II daily followed by UVB irradiation. Petroleum jelly was used here as an inert excipient. All the groups received 2 minutes of irradiation twice daily for first one week. Afterwards all the guinea pigs received irradiation for additional 5 minutes for every additional week till the 8th week.

All the animals were inspected daily for the appearance of erythema which was scored by erythema scale(Figure 2)

(Scale: 0 - No reaction, 1 - Slight erythema, 2 - Moderate erythema, 3 - Severe erythema).[7] After 8 weeks of irradiation and scoring of erythema, the Group I guinea pigs were treated with the isoflavonoid paste daily and Group III guinea pigs were treated with a fine application of tretinoin ointment(0.05%) daily for 4weks. Group I and III animals were further scored for reduction in erythema during this 4weeks. Group II animals were observed for progression of erythema scores. The erythema scores in all the three groups were analyzed by one way ANOVA method.

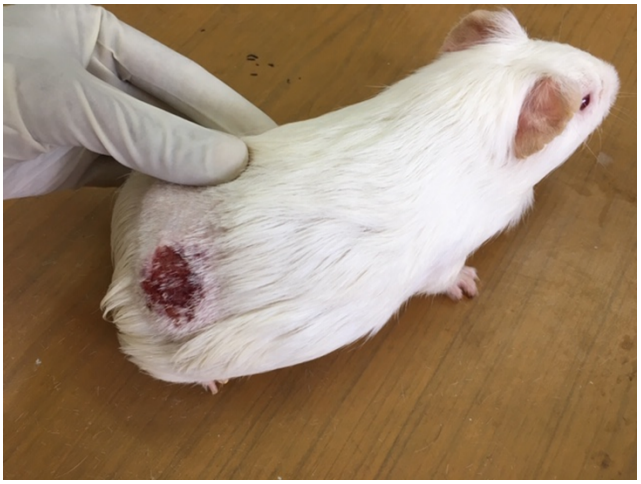

Fig. 2: Guinea pig with erythema

3. Results

After irradiation with UV rays, the mean erythema score at 8^{th} week were measured as 3, 1 and 3 for Group I, II and III respectively (Figure 3).

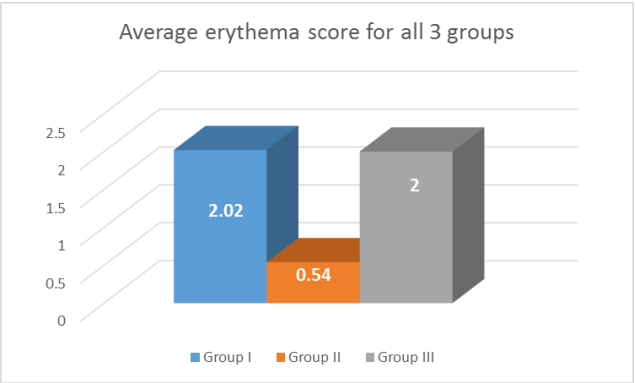

Fig. 3: Average erythema after UV irradiation in all three groups

For guinea pigs of Group II which were pretreated, erythema started appearing only after 4 weeks where as other two groups developed erythema in first week of irradiation. After treatment with isoflavonoids for 4 weeks, Group I recorded change in the erythema score from 3 to 0.). Group III which were treated with tretinoin recorded decline in erythema from 3 to 2.6 at 4^{th} week. Guinea pigs of Group II started having erythema only after 4 weeks which was scored 0.6 and thereafter it increased to 1 in 5^{th} week which persisted up to 8^{th} week and did not increase further. (Figure 4).

Comparison of mean erythema scores by student t test between group I treated with isoflavonoid and Group III treated with tretinoin yielded no significant difference (p=0.5)(Figure 5).

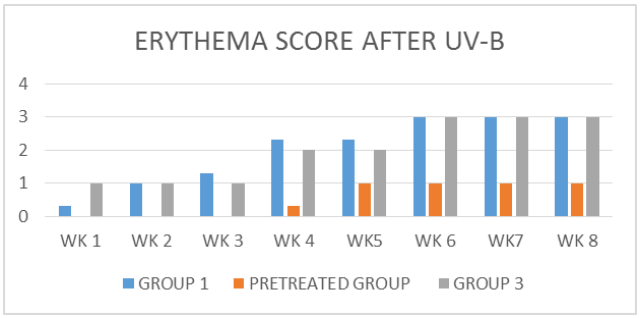

Fig. 4: Comparison of erythema score between three groups of guinea pigs

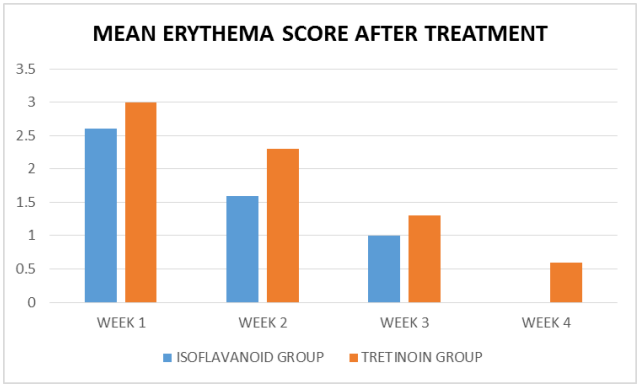

Fig. 5: Post treatment comparison of erythema score between isoflavonoid and tretinoin

4. Discussion

Ultraviolet (UV) radiation, especially UVB with wavelength of 280–320 nm, is one of the major environmental hazards those induce skin damage. The damages to the skin can be in the form of edema, erythema, hyperpigmentation, hyperplasia, photo aging, inflammation or any DNA damage. Review of literature from many studies show that long-term exposure to UV radiation raised the risk of skin cancer due to activation of mitogen-activated protein kinase (MAPK) and p38. More over, UV exposure leads to increased expression of cyclooxygenase-2 (COX-2) that causes cutaneous inflammation and even carcinogenesis. Furthermore, reactive oxygen species (ROS) released as a result of UV exposure, can also add up oxidative stress and skin cell damage.

Many compounds are being used for treatment as well as prevention of UV induced phototoxicity of skin. Of all compounds, Isoflavonoids have been extensively studied for its anti-photo ageing effect. Genistein, one of the isoflavonoids, present in large quantities (1–2 mg/g) in soybeans is known to inhibit pyrimidine dimer formation thereby potently minimizing the detrimental effects of UVB irradiation in UVB exposed human reconstituted skin. Its capacity for regulating these events has been attributed to the free radical scavenging property, the inhibition of photodynamic damage to DNA and the inhibition of tyrosine kinase.[8]

Efficacy of Isoflavonoid in treating phototoxicity has been proved in a study done by Chieh-Chen Huang et al on mice. This study revealed efficacy of soy isoflavone extract Fraction 3 derived from soybean cake in prevention of human keratinocyte apoptosis, attenuation of the level of erythema, reduction in UVB-induced oxidative stress and inflammation of skin cells. It also concluded that soy isoflavone extract is a superior anti-photoaging agent for skin care because of its properties such as no toxicity, easy accessibility, economical feasibility, and environmentally friendly.[9]

In our study, on comparing the mean erythema score between all the three groups, the group that was pretreated with isoflavanoids (Group II) showed late appearance of erythema (only after 4 weeks)than the other groups (Figure 4). This fact proves the prophylactic ability of isoflavonoid against UV induced photo ageing. This finding goes in accordance with the study done by Sun-Young Kim et al. In this study done on hairless mouse models, dietary soy isoflavones caused less wrinkling of the UV-irradiated skin with a concomitant increase in collagen deposition, which is partly due to the inhibitory effects on UV-induced MMP expression and subsequent collagen degradation.[10]

Oh Wook Kwon et al evaluated the protective effects of dietary supplementation with a fermented barley and soybean mixture (BS) on ultraviolet (UV) B-induced photoaging in hairless mice. The BS reduced wrinkle formation, skin thickening, trans epidermal water loss, and matrix metalloproteinases-1 expression in skin. BS has been found to increase superoxide dismutase activity as well as expression of nuclear factor (erythroid-derived 2)-like 2, procollagen type-I which ultimately decrease erythema.[11] The prophylactic effect of isoflavonoids found in this study supports findings of our study in prevention of phototoxicity.

Study done by Y Wang et al has shown that topical application of genistein isoflavone prior to UV-B radiation has reduced c-fos and c-jun expression in the SENCAR mouse skin in a dose-dependent manner. In recent years, genistein has attracted wide attention for its specific inhibition of Tyrosine phosphokinase (TPK) and various anticancer properties. This suppression of UVB-induced proto-oncogene expression in mouse skin suggests that genistein may serve as a potential preventative agent against photodamage and photo carcinogenesis.[12] This finding is in concordance with our finding which adds up to prophylactic effect of isoflavonoid. This study also supports efficacy of topical application of isoflavones.A similar prophylactic effect was elucidated in a study done by Jieun Shin which showed that a combination of Soybean and Haematococcus extract (SHM) prevent UVB-induced skin wrinkling in

hairless mice through matrix metalloproteinases (MMP-1) regulation.[13]

In a placebo-controlled in vivo study done by Kirstin M. Südel, topical application of an isoflavone-containing emulsion was found to enhance the number of dermal papillae per area after 2 weeks. This study concludes as, this soy extract appears to rejuvenate the structure of mature skin.[14] Moreover, Anti-photoaging effect of isoflavone extract from soybean cake has also been proved in the inhibition of UVB-induced apoptosis and inflammation in a study done by Tsu-Man Chiua et al. This study showed that UVB-induced HaCaT cell death and the phosphorylation of p38, JNK, and ERK1/2 decreased in the presence of isoflavone extract.[15]

In our study, on comparing the mean erythema score between isoflavonoids and tretinoin, difference was not statistically significant.(p=0.54)(Figure 5). Though statistically insignificant, the isoflavonoid group revealed much and faster reduction in erythema score than the tretinoin group. Complete nullification of erythema was obtained in 4^{th} week itself. This reduction in erythema by soy iso flavonoids has also been proved by Mitsuyoshi Kano in a study where FSM (Fermented Soy Milk) diet prevented the increase in erythema intensity, epidermal thickness, CPDs, and 6-4PPs in the dorsal skin and in the IL-6 concentration in serum induced by UVB irradiation. Increase in skin thickness is considered a typical response to protect cells such as keratinocytes in the basal layer of the epidermis and fibroblasts in the dermis after UV irradiation. In this study, FSM administration prevented not only skin thickening but also erythema by attenuating inflammation (IL-6 induction) as well as DNA damage.[16] A Systematic Review by Jasmine C. Hollinger et.al has specifically mentioned the double-blind, parallel-group RCT which compared the efficacy of a nondenatured novel soy moisturizer to the vehicle alone in treating 65 women with moderate facial photodamage with promising efficacy.[17]

Adding to the strength of Isoflavonoid in treating phototoxicity, human study has also been done with adorable success. Francesco Bonina et al proved efficacy of soya germ oil(SGO) in reducing erythema in 6 human volunteers after irradiation with UVB. In vivo findings show that SGO possesses a remarkable protective activity against UVB-induced skin inflammation, probably due to its radical-scavenging components, mainly tocopherols and polyunsaturated fatty acids. These results suggest that SGO could find a formidable and important place in therapeutic and cosmetic applications in the management of skin diseases.[18]

Chemo preventative agents function to prevent or alter the various cellular or molecular carcinogenic processes that ultimately lead to tumor growth. Huachen Wei found that Genistein may be used as a topical chemo preventative agent against the adverse effects of UVR on the skin with no side effects. Genistein may be topically applied, alone or co-administered with other medications, to lessen or prevent UVR-induced skin sunburns, premature aging, and skin cancer as it has high lipid solubility and easily penetrates the skin.[19]

Both short term and long term studies have proved topical tretinoin as an effective modality to reduce photoageing. But the most common and frequent adverse effects of topical retinoids especially tretinoin are known as 'retinoid reaction', characterized by pruritus, burning sensation at the sites of application, erythema and peeling due to the free carboxylic acid in the polar end of the retinoid.[20,21] Such adverse reactions were not encountered in our study and in other studies which may be attributed to topical application of isoflavones.[9]

The main limitation of our study was the use of lesser number of guinea pigs for experiment. If these findings can be proved in large number of guinea pigs, we hope this effect can be extrapolated to human population with more success.Using Erythema scale alone as an indicator of photo toxicity or skin damage was the other notable limitation of our study. More than one scale could yield more significant results regarding erythema.

5. Conclusion

The present study has demonstrated the use and efficacy of topical soy isoflavonoids for prevention and treatment of UV induced photo ageing. Use of soy isoflavonoids can be promoted in therapy of phototoxicity over tretinoin with minimal side effects.

6. Conflict of Interest

None.

7. Source of Funding

None.

References

1. Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clin Interv Aging*. 2006;1:327–48.
2. Avci P, Sadasivam M, Gupta A, Melo WCD, Huang YY, Yin R, et al. Animal models of skin disease for drug discovery. *Expert Opin Drug Discov*. 2013;8:331–55.
3. Cho S. The role of functional foods in cutaneous anti-aging. *J Lifestyle Med*. 2014;4:8.
4. Basaria S, Wisniewski A, Dupree K, Bruno T, Song MY, Yao F. Effect of high-dose isoflavones on cognition, quality of life, androgens, and lipoprotein in post-menopausal women. *J Endocrinol Investig*. 2009;32(2):150–5.
5. Vagotis FL, Brundage SR. Histologic study of dermabrasion and chemical peel in an animal model after pretreatment with Retin-A. Springer Science and Business Media LLC; 1995. Available from: https://dx.doi.org/10.1007/bf00451097. doi:10.1007/bf00451097.
6. Mohammed S, Salam NAE, Kalleny N, Bahaa N, Labib J. Effect of Adipose-Derived Stem Cells on Induced Photoaging in the Skin

of Adult Guinea Pig: Histological and Immunohistochemical Study. *Egypt J Histol*. 2017;40(2):184–200.
7. Phototoxicity, In. Sk Gupta; Drug screening methods. *Jaypee Brothers Medical Publishers*;626:629–629.
8. Moore JO, Wang Y, Stebbins WG, Gao D, Zhou X, Phelps R, et al. Photoprotective effect of isoflavone genistein on ultraviolet B-induced pyrimidine dimer formation and PCNA expression in human reconstituted skin and its implications in dermatology and prevention of cutaneous carcinogenesis. *Carcinogenesis*. 2006;27(8):1627–35.
9. Huang CC, Hsu BY, Wu NL, Tsui WH, Lin TJ, Su CC, et al. Anti-photoaging effects of soy isoflavone extract (aglycone and acetylglucoside form) from soybean cake. *Int J Mol Sci*. 2010;11:4782–95.
10. Kim SY, Kim SJ, Lee JY, Kim WG, Park WS, Sim YC, et al. Protective effects of dietary soy isoflavones against UV-induced skin-aging in hairless mouse model. *J Am Coll Nutr*. 2004;23(2):157–62.
11. Kwon OW, Venkatesan R, Do MH, Ji E, Cho DW, Lee KW, et al. Dietary supplementation with a fermented barley and soybean mixture attenuates UVB-induced skin aging and dehydration in hairless mouse skin. *Food Sci Biotechnol*. 2015;24:705–15.
12. Wang Y, Zhang XU, Lebwohl M, Deleo V, Wei HU. Inhibition of ultraviolet B (UVB)-induced c-fos and c-jun expression in vivo by a tyrosine kinase inhibitor genistein. *Carcinogenesis*. 1998;19(4):649–54.
13. Shin J, Kim JE, Pak KJ, Kang JI, Kim TS, Lee SY, et al. A combination of soybean and haematococcus extract alleviates ultraviolet B-induced photoaging. *Int J Mol Sci*. 2017;18(3):682.
14. Südel KM, Venzke K, Mielke H, Breitenbach U, Mundt C, Jaspers S, et al. Novel Aspects of Intrinsic and Extrinsic Aging of Human Skin: Beneficial Effects of Soy Extract¶. *Photochem Photobiol*. 2005;81:581–7.
15. Chiu TM, Huang CC, Lin TJ, Fang JY, Wu NL, Hung CF. In vitro and in vivo anti-photoaging effects of an isoflavone extract from soybean cake. *J Ethnopharmacol*. 2009;126(1):108–13.
16. Kano M, Kubota N, Masuoka N, Hori T, Miyazaki K, Ishikawa F. Oral Administration of Fermented Soymilk Products Protects the Skin of Hairless Mice against Ultraviolet Damage. *Nutrients*. 2016;8(8):514.
17. Hollinger JC, Angra K, Halder RM. Are natural ingredients effective in the management of hyperpigmentation? A systematic review. *J Clin Aesth Dermatol*. 2018;11:28.
18. Bonina F, Puglia C, Avogadro M, Baranelli E, Cravotto G. The Topical Protective Effect of Soybean-Germ Oil against UVB-Induced Cutaneous Erythema: anin vivo Evaluation. *Archiv der Pharmazie*. 2005;338(12):598–601.
19. Wei H. Inventor; Icahn School of Medicine at Mount Sinai, assignee. Genistein as a preventive against ultraviolet induced skin photodamage and cancer. United States patent US 5,824,702. ; 1998.
20. Gilman RH, Buchanan P. Retinoids: literature review and suggested algorithm for use prior to facial resurfacing procedures. *J Cutan Aesthet Surg*. 2016;9(3):139.
21. Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clin Interv Aging*. 2006;1(4):327–48.

Author biography

T Padmavathi Associate Professor

I Israel Raja Johnley Assistant Professor

Prashanth Jeyabaskaran MBBS Student

Cite this article: Padmavathi T, Johnley IIR, Jeyabaskaran P. Efficacy of topical soy Isoflavonoid extract in prevention and reversal of photo-ageing in guinea pig model. *Panacea J Med Sci* 2020;10(2):108-113.