EVALUATION OF EFFECT OF DIFFERENT CONCENTRATIONS OF TITANIUM DIOXIDE ON SUN PROTECTION FACTOR.

Vyawahare NS, Bidkar SG, Shinde SG.

Department of Pharmacology, AISSMS College of Pharmacy, Kennedy Road,

Pune- 411 001, India.

Summary

Sun protection products have been used for mitigating the harmful effects of Ultra Violet (UV) sun rays from time immemorial. These products protects against sunlight induced erythema with the level of performance indicated by sun protection factor (SPF). The present investigation was carried out with the aim to determine the effect of different concentrations of titanium dioxide on SPF using mice. In this investigation the mice were divided into four groups. Group I served as control, Group II and III served as test groups and treated with formulation containing 10% and 20% titanium dioxide respectively. All the mice were anesthetized, shaved 2 X 2 cm^2 area on their back and exposed to UV rays (SPF apparatus) at 850 µ w/cm^2 on 1 x 1cm^2 shaved area and they were periodically observed for burn sign & skin erythema and the UV exposure time required to produce such signs was noted down. The Minimal erythema dose (MED) of exposed area is compared with non exposed area. The results of this study showed that the sample was found to have SPF of the formulation containing 10% titanium dioxide was found to be 6.18 ± 0.14 whereas the SPF of formulation containing 20% titanium dioxide was found to be 14.33 ± 0.25 which indicates that the formulation containing 20% titanium dioxide shows favorable retention time in normal sunlight.

Keywords: sun protection factor, titanium dioxide, Minimal erythema dose, Ultra Violet.

Introduction

Sun burn is the typical ailment in the tropical or moderate climatic countries. Changes in the climate or by sunbathing leads to general increase in daily exposure of skin to ultraviolet (UV) light. Depletion of the stratospheric ozone layer from the most damaging solar UV radiations may also contribute to this increased exposure and further complications like skin cancer[1]. As a consequence the hazards associated with exposure to the UVA and UVB components of sunlight, which include erythema, sunburn, photo damage, photo carcinogenesis, and damage to eyes[2-6]. Phototoxicity is generally characterised by erythema and hyperpigmentation. UVB radiations may cause specific damage to macromolecules, such as DNA, RNA and protein membranes[7]. The usual methods of mitigating these harmful effects are wearing protective clothing, staying out of sun etc. however these methods have their limitations on the other hand the use of sunscreen agents is another method which is becoming popular day by day[8-10]. Sun protection products have been used for mitigating the harmful effects of UV sun rays from time immemorial. These products protect against sunlight induced erythema with the level of performance indicated by sun protection factor (SPF).sun protected products have long protected against sunlight induced erythema with the level of performance indicated by the sun protection factor(SPF). However, since the SPF number is influenced primarily by UVB wavelengths, it is not sufficiently indicator of a sunscreen product's protection against UVA exposure[11].

In recent years, the harmful effects of the UVA wavelengths of sunlight have been more thoroughly established. With this understanding arose a need, not only for sun protection products that were effective against the UVA wavelengths, but also for a common test method for measuring UVA protection levels[11].

Materials and methods

Animals:

Male Swiss albino mice (25±2 g), were procured from the animal house of AISSMS College of Pharmacy, Pune. Mice were placed randomly in polypropylenes cages (six per cage) with paddy husk as

bedding. Animals had free access of standard pellet animal diet and water.

Chemicals and drugs:

Thiopentone sodium injection, Test formulation.

Apparatus:

The SPF apparatus was fabricated as per reported standards. It consists of two wooden compartments (60 x 60 x 140cm). Upper compartment for holding UV radiation source and lower compartment to place animal. Mercury lamp 160 W was used as radiation source to induce erythema. A UV meter was used to measure intensity of irradiation.

Procedure:

The mice were divided into three groups. Group I served as control, Group II and Group III served as test groups and treated with titanium dioxide formulations. All the mice were anesthetized with thiopentone (90 mg/kg/i.p.). A mercury lamp (OSRAM) was warmed up for about 10 minute prior to use and placed at a constant distance (20 cm) above the animal. The intensity was adjusted and stabilised. .The mice were shaved 2 X 2 cm^2 area on their back and exposed to UV rays (SPF apparatus) at 850 µ w/cm^2 on 1 x 1cm^2 shaved area and they were periodically observed for burn sign & skin erythema and the UV exposure time required to produce such signs was noted down. The MED of exposed area is compared with non exposed area. The UV exposure time to produce such sign was noted down. Minimal erythemal dose assessment was carried out by visual (morphological) observation; unprotected and protected skin was observed by same observer[7, 14].

Observations: Intensity of irradiation of radiation measured in µw/cm^2 by using following factor:

$$\text{Intensity of irradiation (J/cm}^2\text{)} = \text{intensity of irradiation (µw/cm}^2\text{)} \times \frac{\text{Time required to Produce erythema (min.)}}{1.66}$$

Table no 1: Time required to produce erythema in mice.

Sr no.	Time required to produce erythema			
	Control	Standard	WL-1(10%)	WL-2(20%)
1	9	140	65	151
2	8	145	60	148
3	11	128	60	145
4	12	130	67	142
5	10	132	62	140
6	11	137	63	146
Mean ±SEM	10.16 ±0.6009	135.33 ± 2.654***	62.83 ± 1.138***	145.33 ± 1.626***

Values are expressed as Mean ± SEM (N=6)

***The P value is < 0.001, considered significant.

Calculation of SPF:

Formula:

$$SPF = \frac{\text{Minimal erythema dose in Test formulation protected skin in j/cm}^2}{\text{Minimal erythema dose in Non sunscreen protected skin in j/cm}^2}$$

Results

MED was evaluated in normal grouped animals and accordingly the standard, WL-1 and WL-2 group. Mice were exposed to the UV radiation fixed at 850µw/cm$^{2.}$ The average time was calculated to be 9 min 30 sec for producing erythema. The

morphological evaluations of the skin of standard, WL-1, WL-2 were found to be similar. From the observed time to produce skin burn the MED was evaluated for SPF calculation. The SPF of standard was found to be 13.58 ± 0.80.however the same of WL-1, WL-2 were found to be 6.18±0.1471 and 14.33±0.2540 respectively.

Table no.2: Sun Protection Factor.

Animal No.	Sun Protection Factor		
	Standard	WL-1(10%)	WL-2(20%)
1	14.01	6.50	15.11
2	14.51	6.00	14.81
3	12.81	6.00	14.51
4	13.01	6.70	14.21
5	13.2	6.2	14.00
6	12.45	5.72	13.36
Mean ± SEM	13.33±0.3172	6.18±0.1471	14.33±0.2540

Discussion

The sun protection test is used specifically to evaluate the sunscreen agents. The sun protection test measures ability of sunscreen to protect skin from sun radiation. The standard formulations exert their sun protection effect via blocking the UV radiations. Exposure to the UVA and UVB components of sunlight, which include erythema, sunburn, photo damage, photo carcinogenesis, and damage to eyes[2-6]. In sun protection test WL-1 and WL-2 showed significant ($p \leq 0.05–0.001$) and dose dependant sun protection action. From the results it can be inferred that the Test formulation (WL-2) was found to have SPF of 14.33 ± 0.25 and shows moderate retention time in normal sunlight.

References

1. Abarca JF, Casiccia CC. Skin cancer and ultraviolet-B radiation under the Antarctic ozone hole: southern Chile, 1987-2000. Photodermatol Photoimmunol Photomed. 2002 Dec; 18 (6):294-302.

2. Drobetsky EA, Turcotte J, Chateauneuf A. A role for ultraviolet A in solar mutagenesis. Proc Natl Acad Sci U S A. 1995 Mar 14; 92(6):2350-4.

3. Taylor CR, Stern RS, Leyden JJ, Gilchrest BA. Photoaging/photodamage and photoprotection. J Am Acad Dermatol. 1990 Jan; 22(1):1-15.

4. Lucas RM, Ponsonby AL. Ultraviolet radiation and health: friend and foe. Med J Aust. 2002 Dec 2-16;177(11-12):594-8.

5. Clydesdale GJ, Dandie GW, Muller HK. Ultraviolet light induced injury: immunological and inflammatory effects. Immunol Cell Biol. 2001 Dec; 79(6):547-68.

6. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer Journal of Photochemistry and Photobiology B: Biology 63 (2001) 8–18

7. Nicolas JL, Nadim AS, Madhu AP. Sunscreens,developement,evaluation and regulatory aspects .cosmetic science and technology series, volume 15,second edition ,Marcel Dekker, New York, page no. 499-512

8. Thompson SC, Jolley D, Marks R. reduction pf solar keratoses by regular sunscreen use. N.Engl.J.Med. 1999; 329,1147-1151

9. Diffey BL, Robson J. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. J. Soc.cosmet.chem., 40; 127-133.

10. Tatiana A, Elisabetta D, Maurizio B,Lucedio G,Giovanni P. A lack of in vitro protection by a common sunscreen ingradient on UVA- induced cytotoxicity in keratinocytes. Toxicology 203; 165-178.

11. Notes of guidance for testing of cosmetic ingredients for their safety evaluation. Third revision the scientific committee on cosmetic products and non food products intended for consumers.1999

12. Method for the in vitro determination of UVA protection provided by sunscreen products, Guideline 2007. Prepared by the colipa in vitro photoprotection methods task force.

13. Pissavini M, Fererro L. in vitro determination of sun protection factor. Business briefing 1-5

14. Australia/New Zealand sunscreen standard. AS/NZS 2604.1998 SPF test method. Comparative summary.2004.