Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 1
ISSN: 2321–3647(online)

Efficacy of Oral Ocimum Sanctum in Prevention of Uv Induced Photoaging in Mice Model

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ABSTRACT

Photoaging is the term used to describe the characteristic changes that appear in the skin when it is chronically exposed to Ultra Violet (UV) rays. Photoaging is associated with increased risk of causing skin cancer. Antioxidants play an important role in prevention and treatment of photoaging. To evaluate the preventive efficacy of oral Ocimum sanctum against photoaging in mice model. Six weeks old Swiss Albino mice of either sex weighing 25g to 35g were divided into three groups A, B, C (n=6). Group A was given UV irradiation alone, group B was given UV irradiation and oral Ocimum sanctum, group C was neither given UV irradiation nor oral Ocimum sanctum, for 8 weeks. Groups A, B and C were sacrificed and evaluated for moisture content, epidermal thickness by histopathology and antioxidant property by TBARS assay. Results were expressed as Mean ± SEM. Statistical analysis were done by One way ANOVA and student's unpaired t test. Efficacy in prevention: Moisture content of group A (11.57±0. 29) showed significantlylower value than group B (21.96 ±2. 1). Group B has a lower value compared to group C (27.56 ±2. 4). On TBARS assay, Group A (1.083±0.03) value is more than group B (0.459±0.01), Group B value is more than group C (0.536±0.01). On histopathology examination group A showed atrophy of epidermis. B showed near normal histology and C showed normal histology. This study has proved that oral Ocimum sanctum can be used for prevention of photoaging. It warrants further study in humans before including them in the prophylaxis of photoaging.

Keywords: Photoaging, Ocimum sanctum, UV irradiation.

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Received 17 December 2014, Accepted 19 January 2015

Please cite this article as: Femijas MP *et al*., Efficacy of Oral Ocimum Sanctum in Prevention of Uv Induced Photoaging in Mice Model. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

Photoaging is a phenomenon characterized by specific changes that appear in skin when chronically exposed to Ultra Violet (UV) rays. The term 'photoaging' was first used twenty eight years ago, though the concept was in existence much earlier[1]. By the 19th century, the difference between the skin of farmers and outdoor workers and those who worked indoors was noted by many; increased incidence of skin cancers among outdoor workers was also pointed out[1]. Photoaging although usually benign, is associated with an increased risk of skin cancer, leading to mortality[2]. As per reports, around 66,000 deaths per year occur due to skin cancers all over the world which has led to a significant number of studies in the field of photoaging[3]. Another important reason for interest in the field of photoaging is cosmetic concern, a large market worth over 12 billion US dollars and growing[4]. Thirdly, incidence of photoaging is increasing exponentially as the human exposure to environmental UV rays is increasing primarily due to depletion of the ozone layer[2]. An important reason for increased exposure to UV rays is the widespread belief that sunscreen protects completely from UV rays which led to increased duration of exposure to UV rays by promoting a false sense of security for consumers[5]. Prolonged life expectancy leads to increased exposure to UV rays. Longer traffic transit time has also risen as a contributor for increased UV ray exposure, especially in countries like India where UV protective window screens on vehicles are banned[5,6]. Changes in lifestyle, like the increased importance of outdoor leisure activities and travel to equatorial regions involving UV exposure are other factors[5]. Apart from environmental UV rays, use of artificial UV rays like tanning parlors has resulted in an increased incidence of photoaging[6]. Photoaging has multiple etiologies, precipitating and causational factors which can be divided in to biological (age, gender) and ecological factors. Ecological factors are sun rays, chemical pollutants and lifestyle factors like cigarette smoking, and nutrition[7]. Among the sun rays, UV rays are the predominant contributor for photoaging though infrared and visible spectrum partly contribute[8]. Pathology behind induction of photoaging due to UV rays are oxidative stress and mutation of mitochondrial DNA[9]. Oxidative stress is a result of reactive oxygen species (ROS)[6]. This ROS and oxidative reaction can be controlled by using antioxidants. Taking account of the fact that photoaging is partly but significantly due to oxidative reaction of various reactive species, we planned to study Ocimum sanctum which is commonly known as Tulasi in India for their potential antioxidant properties[7].

MATERIALS AND METHOD

Healthy Six weeks old Swiss Albino mice of either gender weighing between 25 g and 35 g were procured from King's Institute, Guindy, Chennai (CPCSEA registered breeder) and maintained in Central Animal House of our institute after getting approval from the Institutional Animal Ethics Committee. The mice were housed in polycarbonate cages with cut-straw bedding and fed with standard chow and water *ad libitum*. A 12:12 hour dark and light cycle was maintained in the animal house as per CPCSEA guidelines. Eighteen mice of either gender of weight 25g to 35g older than six weeks were acclimatized for two weeks, they were weighed and the values were noted. The mice were then divided randomly into three groups (A, B and C) with six mice in each. Group A received UV irradiation and normal diet (positive control), Group B received UV irradiation and oral Ocimum sanctum (40mg/kg bodyweight once daily, obtained from from Himalayan drug company, Bangalore), (test group). Group C received normal diet, neither UV irradiation and nor Ocimum sanctum (negative control).

Table 1: Grouping of mice

Groups	**For 8 weeks**		
Group a	Normal diet	UV irradiation	Data analysis
Group b	Normal diet+ Oral Ocimum sanctum. (40mg/kg/day)	UV irradiation	
Group c	Normal diet	No UV irradiation	

After randomly assigning the mice into groups, an area of 4 cm^2 was marked on the dorsal surface of the mice and a depilatory cream (commercial preparation *Anne French*) was applied as directed in the product usage sheet and hair was removed from the marked area. The animals were subsequently observed for 48 hours for any reaction to the depilatory cream. UV irradiation was administered using a UV lamp (Ultra Vitalux300WOsram light capable of both UV-A/B emission was obtained from Variety Lighting Equipment Pvt Ltd, Bangalore, India) that provides the full spectrum of radiation simulating the Solar Radiation, i.e., 260 – 400nm[8]. The amount of irradiation was controlled by the duration of exposure. The mice in the irradiated groups received two minutes of irradiation twice a day (total 4mins) for the first one week, and were received five additional minutes for every additional week till the eighth week[8]. Animals were fed with Ocimum sanctum(40mg/kg bodyweight once daily) and normal diet using steel gavage tubes[7].After eight weeks, groups A, B and C were sacrificed by cervical dislocation under Sodium pentobarbitone anaesthesia as per method of Urikura, *et al*[9]. Immediately thereafter, the skin strips of size 4cm^2 were cut antero-posteriorly using a scalpel under strict aseptic precaution.

Skin was again cut in to three pieces of size 1cm^2, 1cm^2 and 2 cm^2 for weight measurement, histopathology and TBARS assay, as per the method of Sachideva, *et al.*, Agarwal and Urikura I[8,9,10]. Skin which was cut into 1 cm^{2}strips was weighed immediately to get the wet weight of the skin, then it was kept for 24 hours and weighed again to get the dry weight of skin. And then the moisture content was calculated by using the following formula as described by Sachideva, *et al*[8].

$$\text{Moisture content in \%} = \frac{wet\ weight\ of\ skin - dry\ weight\ of\ skin}{wet\ weight\ of\ skin} * 100$$

TBARS of skin was done in Research Lab of Department of Biochemistry, MGMCRI, Puducherry. Absorbance was determined at 535nm against a blank that contained all the reagents minus the skin homogenate in a spectrophotometer (UV-5200/5300/5500 spectrophotometer). Malondialdehyde concentration was calculated using the extinction coefficient of 1,56,000 m^{-1}cm^{-1}[,9]. The cut skin specimen was immediately transferred into 10% N formalin and histopathology examination was performed in the lab of Department of Pathology, MGMCRI, Puducherry. Collected data were entered and analysed using Microsoft Excel 2013. Results were expressed in Mean ± SEM as tables. Statistical analysis was performed using One Way ANOVA followed by student's unpaired t test. The P value < 0.05 was considered as statistically significant at 95% confidence interval.

RESULTS AND DISCUSSION

The study was conducted in the department of Pharmacology in collaboration with Department of Biochemistry and Pathology at Mahatma Gandhi Medical College and Research Institute, Puducherry. Effect of Ocimum sanctum in prevention of photoaging were accessed using three parameters, moisture content, TBARS assay and histopathology. On calculating percentage of Moisture content ofskin of mice[8] we obtained the following results as shown in figure 1. The antioxidant property of mice was estimated by TBARS assay. In this assay metabolite of lipid peroxidation (Malondialdehyde) combined with ThioBarbituricacid to form the Barbituric Acid reactive species which was then read spectrophotometricaly at 535nm concentration calculated using an extinction coefficient of 1,56,000M^{-1}cm^{-1}and expressed in µmol/g[9]. The first parameter measured was moisture content percentage calculation. Moisture holding capacity of mice which were UV irradiated and treated simultaneously with oral Ocimum sanctum was better than that of mice which were UV irradiated and not given oral Ocimum sanctum. Moisture holding capacity depends on laxity and wrinkling of skin[8]. In UV irradiated mice,there is increased production of ROS which in turn increases MMP. This enzyme increases collagen breakdown there by

decreasing skin laxity and wrinkling of skin[11]. This skin laxity and wrinkling are reduced by antioxidants which reduce ROS production by preventing oxidative reaction[6]. In our study, the group which received only UV irradiation had shown less moisture holding capacity than the group which received Ocimum sanctum followed by UV irradiation. This implies that Ocimum sanctum have some role in decreasing skin laxity and wrinkling. These findings are in accordance with a study done by Sachdeva, *et al.* in India with an antioxidant *Prunus Amygdalus* commonly known as almond[8]. The group which received both oral Ocimum sanctum and UV irradiation (group B) was compared with a group which has neither received UV irradiation nor do oral Ocimum sanctum (group C). Group C was not having any significant difference in water holding capacity than the group B. This resultimplies that group B has no wrinkling and laxity of skin, this finding is also in accordance with a study done by Sachdeva, *et al* in India[8].

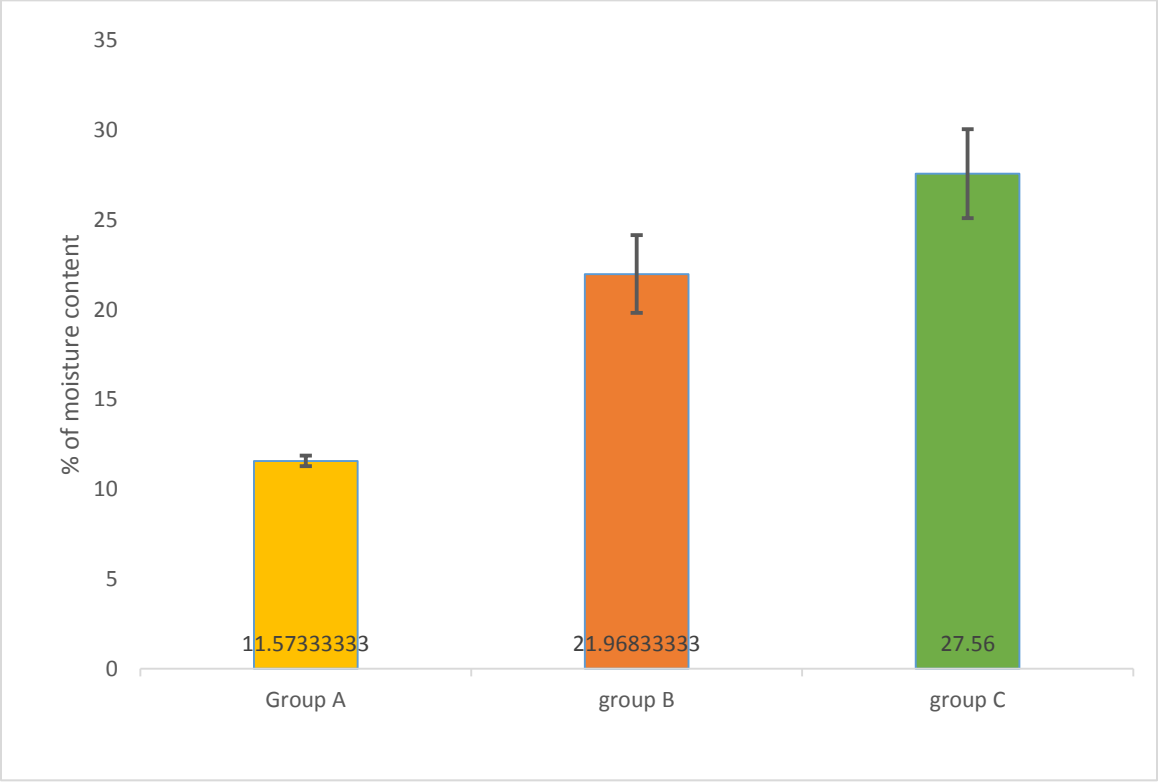

Figure 1: Percentage of moisture content in prevention of photoaging

All the values are expressed as mean ± SEM with n=6, Significant P value (<0.05) with One way ANOVA, when test Group (Group B) (21.96±2.16) and positive control (Group A) (11.57±0.29) was compared with the negative control group (Group C) (27.56±2.47).

Significant P value (<0.05) with student's unpaired t test when group A compared with group B.

Significant P value (<0.05) with student's unpaired t test when group A compared with group C.

P value (>0.05) not significant with student's unpaired t test when group B compared with group C.

TBARS value in prevention of photoaging

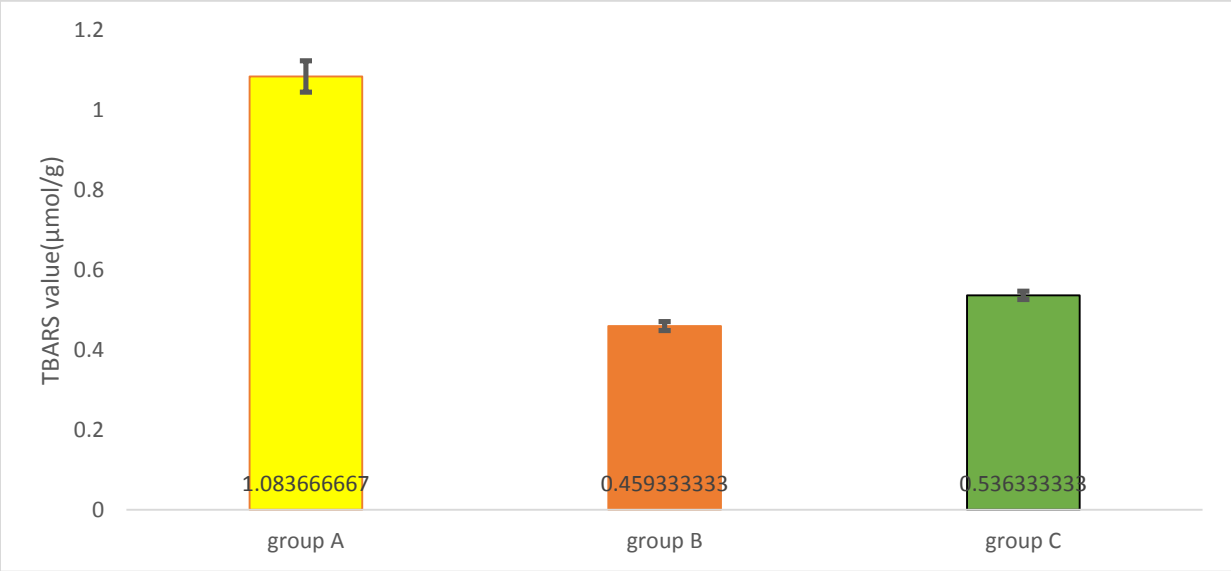

Figure 2: TBARS value in prevention of photoaging.

All the values are expressed as mean ± SEM with n=6. Significant P value (<0.05) with One way ANOVA, when test Group (Group B)(0.45±0.01) and positive control (Group A) (1.08±0.03) was compared with the negative control group (Group C) (0.53±0.01).

- Significant P value (<0.05) with student's unpaired t test when group A (positive control group) is compared with group B (test group).
- Significant P value (<0.05) with student's unpaired t test when group A (positive control group) compared with group C (negative control group).
- Significant P value (<0.05) with student's unpaired t test when group B (test group) compared with group C (negative control group).

Effect on Skin TBARS Level, TBARS value gives the value of a metabolite named Malondialdehyde of oxidative reaction, so when there is a rise in TBARS value it indicates increased concentration of Malondialdehyde due to increased oxidative reaction[9]. In our study group A which was given UV irradiation without any medication, there was higher TBARS value than other groups. This indicates that UV irradiation has produced some oxidative stress resulting in increased lipid peroxidation of skin, this led to rise of Malondialdehyde which in turn increased TBARS value[12]. Group B which received UV irradiation immediately after oral Ocimum sanctum had TBARS value less than group A. Thus, Ocimum sanctum reduced lipid peroxidation of skin by effective quenching of singlet oxygen, thereby decreasing the production

of MDA. When group C (neither UV irradiated nor given any Ocimum sanctum) was compared with Group B, group C had lesser value of TBARS which may be due to less oxidative reaction as it was not exposed to UV irradiation, and the low value of TBARS indicated that there is some natural oxidation reaction going on apart from that induced by UV irradiation. Results of TBARS in prevention of photoaging with oral Ocimum sanctum are in accordance with a study done by Urikura,*et. al* with fucoxanthin a Carotenoid as topical agent for prevention of UV induced photoaging[9]. In the study by Sachdev, *et al* .using *Prunus Amygdalus* in abatement of detrimental effects of photoaging, the researchers have shown an increase in Malondialdehyde, results were similar with increased level of MDA in only UV irradiated group and lower value in group treated with *Prunus Amygdalus* followed by UV irradiation[8]. Our study results arealso comparable to findings in studies by Reeve EV,*et al.* with Isoflavone Eqoul which has antioxidant property and act as protective agent in photoaging[12].

Histopathological examination of skin

Histopathological examination of skin showed following features Group A (received UV irradiation without oral Ocimum sanctum) has thinning that is atrophy of epidermis, collagenisation of dermis, attenuation of adnexal structures and mild inflammatory infiltrates which is suggestive of impact of UV irradiation

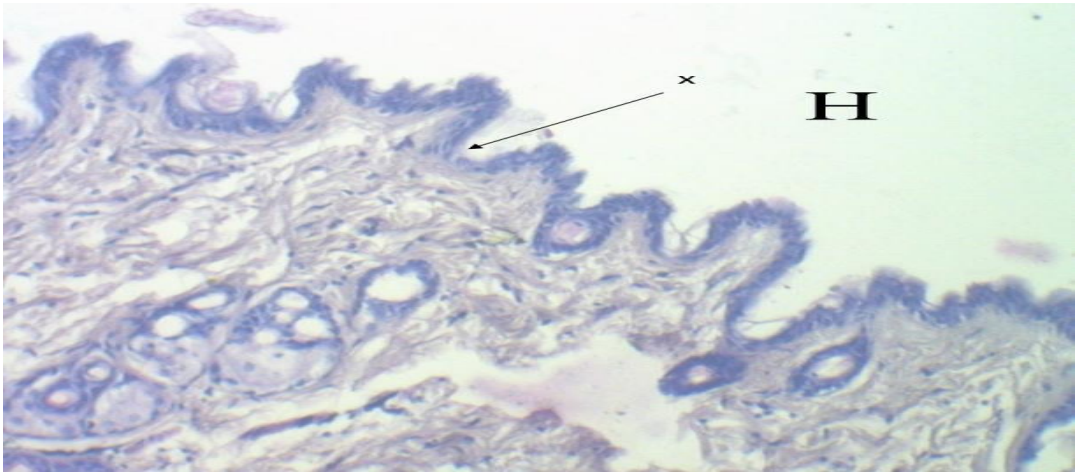

Figure 3: Histopathology of group A:Skin sections were stained with eosin and hematoxylin. (H) Group A, arrow 'X' indicates atrophy of epidermis. (Magnification, 10X)

Group B (oral Ocimum sanctumsfollowed by UV irradiation) has a variable response with one slide showing near normal features of epidermis and dermis. Two slides showing partly focal atrophy and partly focal regeneration and another two slide showed only atrophy, altogether, suggesting that some changes of regeneration have occurred in the majority of the slides indicating that oral Ocimum sanctum as preventive strategy has some beneficial effect

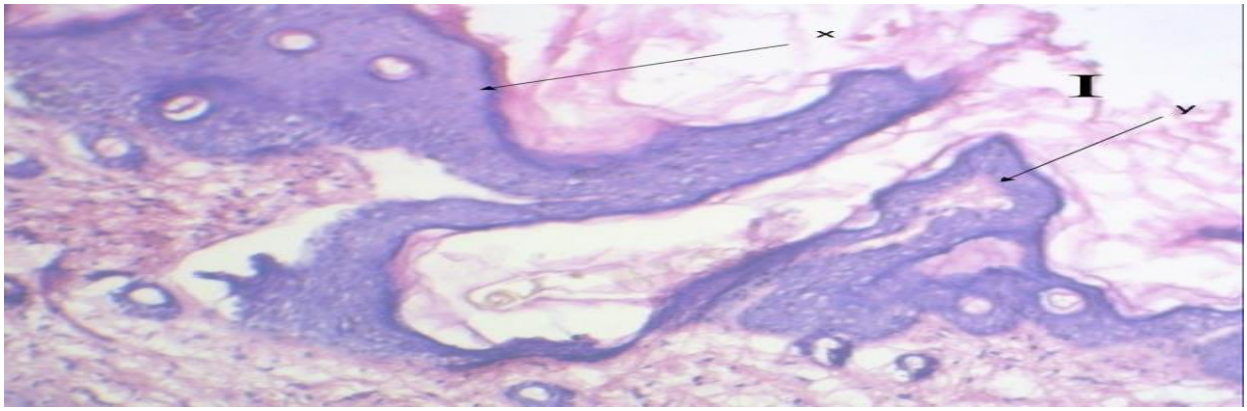

Figure 4: Histopathology of group B:Skin sections were stained with eosin and hematoxylin. (I) Group B, arrow 'y' showing areas of epidermal atrophy and arrow 'X' showing epidermal regeneration as well. (Magnification, 10X)

Group C (neither UV irradiation nor Oral Ocimum sanctum) all the slides showed normal epidermis and dermis.

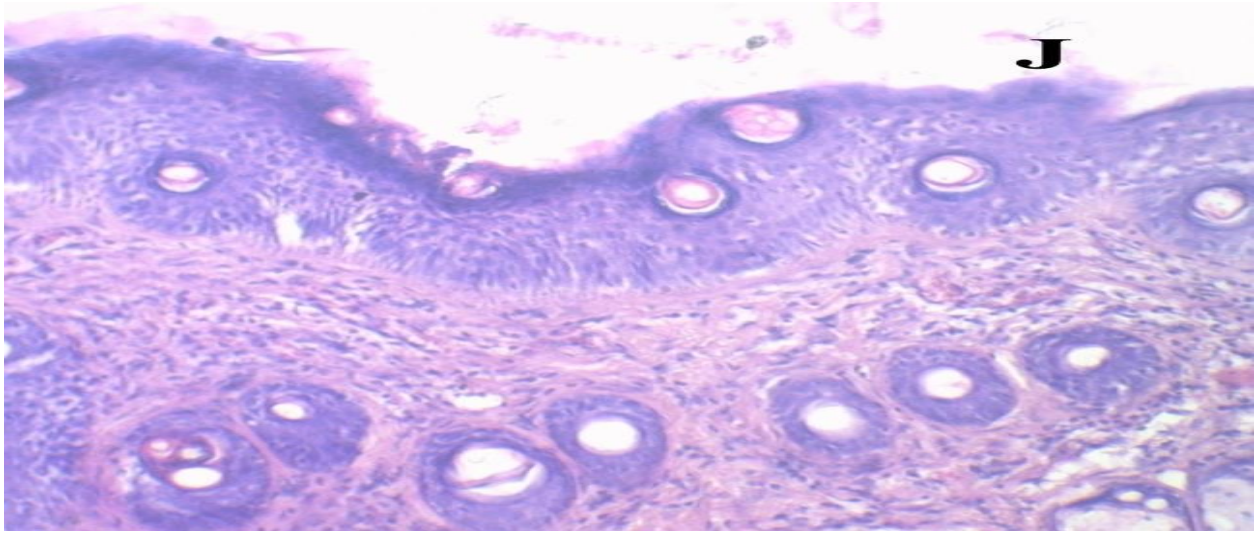

Figure 5: Histopathology of group C:Skin sections were stained with eosin and hematoxylin. (J) Group C has normal epidermis. Magnification, 10X.

Effect on Histopathology of skin, on histopathological examination, the group which received only UV irradiation has shown atrophy of the epidermis with collagenisation of the dermis. Attenuation of adnexal structures and mild inflammatory infiltrates was seen, which is a typical sign of UV irradiation as per Lever's Histopathology of Skin[13]. When skin is exposed to UV rays, UV rays produce ROS which in turn activate AP1, which activates MMP. This MMP will degrade collagen and elastin fibers leading to atrophy of skin[6]. Group B which had received oral Ocimum sanctum followed by UV irradiation showed variable result with one slide showing near normal skin which looks absolutely conclusive finding. Two slides have shown some areas with

atrophy of dermis-a sign of UV induced damage and some areas with normal dermis indicating that oral Ocimum sanctum has protective effect on skin. Some areas with a sign of regeneration suggest that they are under repair by oral Ocimum sanctum. Over all, there is a protective effect for oral Ocimum sanctum. Lack of complete normality of skin may be due to lesser duration of the study period or a decreased dose. When skin is subjected to UV rays, it activates ROS, this in turn activate AP1, which activates MMP. This MMP will degrade collagen and elastin fibers resulting in atrophy of skin. So when we are using Ocimum sanctum a potential quencher of ROS, there is no activation of AP1 and atrophy of skin is prevented by this probable mechanism[14]. This finding is in line with the study done by Agarwal and Kaur with an antioxidant agent Curcumin in prevention of UV induced photoaging of skin[10]. Group C which received neither oral Ocimum sanctumnor UV irradiation has shown normal epidermis and dermis with adnexal structures as per features given in Lever's Text Book of Histopathology of Skin[13]. These results obtained clearly showed the involvement of oxidative reaction induced by UV rays in the pathogenesis of photoaging of skin. This study also indicates the effect of oral Ocimum sanctum in prevention and reversal of photoaging by its antioxidant action. Although our study showed that Ocimum sanctum are effective in preventing, much more studies are required with large samples, hairless mice and advanced investigative procedures like Stephens Wrinkle Imaging using Raking Light (SWIRL), which analyzes the severity of wrinkles using photograph[15]. Evaluation of water holding capacity could have been better if had used standard water content examination technique like 3c system Dermotech, which is a computerized tool access skin hydration[16]. Histopathology examination would have been more accurate if it had measured the epidermal thickness using optical coherence tomography[17]. These are some of the limitations of our study.

CONCLUSION

In this present study, effectiveness of Ocimum sanctum administered orally for prevention of photoaging by its antioxidant property was assessed by moisture content of skin, biomarkers of oxidative stress and histopathology of skin. We found that Oral Ocimum sanctumhas significant beneficial effects in prevention, but further studies preferably in humans are needed to confirm that oral Ocimum sanctum can be routinely administered for prevention of photoaging.

ACKNOWLEDGMENTS

Our study dint receive any financial support. Technical support was given by department of Biochemistry and Pathology.

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