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Research Article

Photo-protective Effect of *Murraya koenigii* (Curry Leaf) Against Photodamage Induced in Swiss Albino Mice Under Acute Exposure to UVB Radiation.

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ABSTRACT

Exposure of the skin to UVB radiation causes skin damage. UVB is considered lethal as it spontaneously causes sunburn, is genotoxic, and responsible for significant depletion of dermal antioxidants (enzymic and non-enzymic). It initiates photochemical reactions resulting in extensive free radical generation and in turn oxidative stress. The harmful effects of chemical based commercial sunscreens has led to the exploration of "safe" and "economical" alternatives. One approach is to use plant antioxidants as potential photoprotectives. Recent years have witnessed the application of antioxidative phytochemical compounds such as phenolic acids, flavonoids and high molecular weight polyphenols, as beneficial photoprotective agents. However, the photoprotective role of commonly available antioxidant rich aromatic plants such as *Murraya koenigii* (Curry Leaf) is still less understood. In the present study, antioxidant activity of *Murraya koenigii* extracts was studied qualitatively and quantitatively. Hot and Cold chloroform extracts of *M.koenigii* showed maximum antioxidant activity with IC_{50} values of 17.46 µg/µL and 16.06 µg/µL respectively. Dermal antioxidant enzymatic response to a single UVB exposure was studied in *Swiss albino* mice by determining the change in catalase and superoxide dismutase enzyme activities in the presence and absence of the plant extract. In the absence of the plant extract, the antioxidant activity of enzymes SOD and CAT decreased significantly ($p<0.05$) indicative of the stress induced. SOD enzyme showed a significant ($p<0.05$) improvement in its activity in the presence of the plant extract. The study thus indicates the photo-protective effect of cold chloroform extract of *M.koenigii*, against acute UVB damage induced in *Swiss albino* mice.

Keywords: UVB; Ultra violet radiation; photo-protective effect; Murraya koenigii; Curry Leaf; Superoxide dismutase; SOD; Catalase; CAT; MED, antioxidant activity, DPPH radical scavenging assay, Native PAGE.

INTRODUCTION

As a consequence of the depleting ozone layer, recent years have witnessed an alarming rise in skin disorders caused as a result of incoming solar radiation, which is continuing to grow[1,2]. UV radiation is a potent initiator of photochemical reactions responsible for free radical generation in the exposed surface. The major acute effects of UV irradiation include sunburn, inflammation (erythema), tanning, histopathological changes such as thickening of the epidermis, local/systemic immunosuppression and DNA damage at the molecular level. Chronic exposure leads to photoaging, sustained immunosuppression, and ultimately photocarcinogenesis[3,4]. Solar UV radiation comprises the electromagnetic spectrum from 200 to 400 nm. It is further subdivided into UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm), each having distinct biological effects based on its energy (intensity) and penetration power. UVC is blocked from reaching the Earth's surface by the stratospheric ozone layer. UVA and UVB both reach the Earth in sufficient amounts, accounting for important biological consequences caused to the skin and the eyes[4,5]. Exposure to UVB radiation stimulates free radical generation thus decreasing or impairing the ROS scavenging systems (enzymic and nonenzymic) in the skin. Chemical based sunscreens have long been used as photo-protectants against UV damage at the molecular and tissue level. Several of them have been proved to be mutagenic, several others being responsible for cutaneous reactions[6]. Numerous studies involving the use of natural antioxidants (vitamins, beta-carotenes) as substitutes to sunscreens have been investigated[7]. Although they did show good results at the pre-clinical level, clinical evidence of them being potential photo-protectors against solar radiation was found to be unconvincing[7,8]. Anti-oxidant compounds such as phenolics, extracted from various plant sources were studied and confirmed as potential candidates *in*

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vitro and *in vivo*, for preventing the adverse effects of UV radiation experiments[9-12]. There have also been studies carried out to understand the relationship between UV radiation and enzymatic antioxidants in the skin. These reports claim that UV radiation decreases the activities of antioxidant enzymes such as SOD, CAT and GPX[13-16]. Curry leaf (*Murraya koenigii*) extract, among compounds under investigation; has been reported to be extremely rich in polyphenolic content exhibiting potent free radical scavenging activities[17-19]. This is the first report of the series describing some aspects of oxidative stress induced in Swiss albino mice on acute exposure to UVB and recovery from stress by using a phytochemical extract.

MATERIALS AND METHODS

Chemicals

Solvents methanol, chloroform, hexane, hydrogen peroxide, toluene, ethyl acetate, formic acid, hydrochloric acid and sodium carbonate were purchased from Qualigens Fine Chemicals, Fisher Scientific, India. DPPH was purchased from Sigma Aldrich, St. Louis, USA. Silica Gel GF-254 (100-200 mesh size), Copper (III) sulfate pentahydrate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, potassium ferricyanide, ferric chloride and ammonium persulfate were obtained from Merck Chemicals, India. Ascorbic acid, pyrogallol, riboflavin and bovine serum albumin were procured from Loba Chemie, India. Ethanol was purchased from Changshu Yangyuan Chemical, China. Tris base, Nitroblue tetrazolium (NBT) and Folin-Ciocalteaue reagent were obtained from Sisco Research Laboratories, Pvt. Ltd., India. EDTA, acrylamide, bis-acrylamide and TEMED were acquired from S.D. Fine Chem Ltd., India.

Plant Material

Leaves of *Murraya koenigii* (Curry Leaf) were obtained from local fields at Palghar region, in the outskirts of Mumbai, Maharashtra, India. They were acquired in the months of December to January, 2009. For the study, the dried herbarium of *M. koenigii* was authenticated and deposited at the Sebato Herbarium, St. Xavier's College, Mumbai (Accession no. 8910). Two thousand grams of curry leaves were shade dried at ambient temperature (28-30°C) for 15-20 days until complete dryness. The dried leaves were coarsely ground using a domestic electric grinder and stored in clean, dry containers away from light until further use. Herbal base (shata-dhauta-ghruta) used to dissolve the plant extract was procured from a regular Ayurvedic Medical Store in Mumbai, India.

Phytochemical Extraction

Phytochemicals were extracted using hot as well as cold Soxhlet extraction methods. Hot extraction was carried out by adding 50 grams of the coarsely ground Curry leaf powder to 300 ml each of chloroform, Methanol, Hexane, and methanol: water (1:1). Cold extraction was carried out by adding 25 grams of the coarsely ground Curry leaf powder to 125ml each of methanol, chloroform, hexane, methanol: water (1:1). Phytochemical extraction was carried out on an orbital shaker (120 rpm, room temperature) for 48 hrs. Cold extraction method was used for the extraction of heat-labile compounds. Post solvent-extraction, the solvents were completely removed by air-drying at room temperature. Aqueous extracts were dried at 40^{o}C. Percentage yield of each extract was calculated and stored in the refrigerator in clean, dark-bottles till further analysis.

Stability of the Plant Extracts

Chemical stability of the extracts was determined at 0 and 6 monthly interval spectroscopically[20] through V-550 UV-Vis spectrophotometer (Jasco, Japan) and FTIR 460-Plus spectrophotometer (Jasco, Japan). 1:500 and 1:1000 dilutions of the plant extract (1mg/mL) were used for determining stability of the plant extract.

Determination of Total Anti-oxidant content

The antioxidant activity of the extracts was determined qualitatively and quantitatively using stable DPPH (1, 1 diphenyl 2, picryl hydrazyl) radical as per the method proposed by Sanchez –Moreno[21].

Qualitative analysis

For thin layer chromatographic separation of the plant solvent extracts, the extracts were spotted on the TLC plate (20 µg/µl) and separated using mobile phases toluene: ethyl acetate (9:1) for hexane plant extracts and toluene: ethyl actetate: formic acid: water (10:1:1:1) for methanol, chloroform, and aqueous plant extracts. The chromatogram was allowed to develop for 30 minutes and then sprayed with DPPH reagent (0.15 % w/v in methanol). The color change (yellow color development against pink-purple background on the TLC plate) was noted as an indicator for the presence of antioxidants[22].

Quantitative analysis

The free radical scavenging capacity of the extracts was determined spectroscopically using DPPH reagent. 2.0 ml reaction mixture contained 40 µM of freshly prepared DPPH solution in methanol and 10 µl of each plant extract (10mg/ml). Absorbance at 517 nm was recorded at intervals of 1 minute until 5 minutes and then at 30th minute using V-550 UV-Vis spectrophotometer (Jasco, Japan). Total antioxidant content of the plant extracts was represented as $IC_{50} \pm SE$ using ascorbic acid as the reference standard. Control sample of DPPH was prepared containing the same volume without any extract and 95 % methanol served as the blank[22-24]. The IC50 value of the plant extracts was calculated and compared with ascorbic acid used as the positive control. IC50 value denotes the concentration of plant sample required to scavenge 50% of DPPH free radicals.

Animals and UVB irradiation

Mixed population of male and female *Swiss Albino* mice with average weight between 28-33 grams, were obtained from the Animal House at the Haffkine Institute for Training and Research, Mumbai, India. The animals were disease free maintained in clean, dry, polypropylene cages, at a room temperature of 25^{o}C, humidity 60-65%. They were exposed to natural day-light cycles. Standard pelleted rodent feed (Lipton India Ltd.) was provided *ad libitum*. The animals were euthanized by chloroform inhalation prior to tissue excision. A 12" UVB lamp (Sankyo Denki, Japan) with an emission spectra of 280 – 320 nm peaking at 306 nm and UVB output wattage of

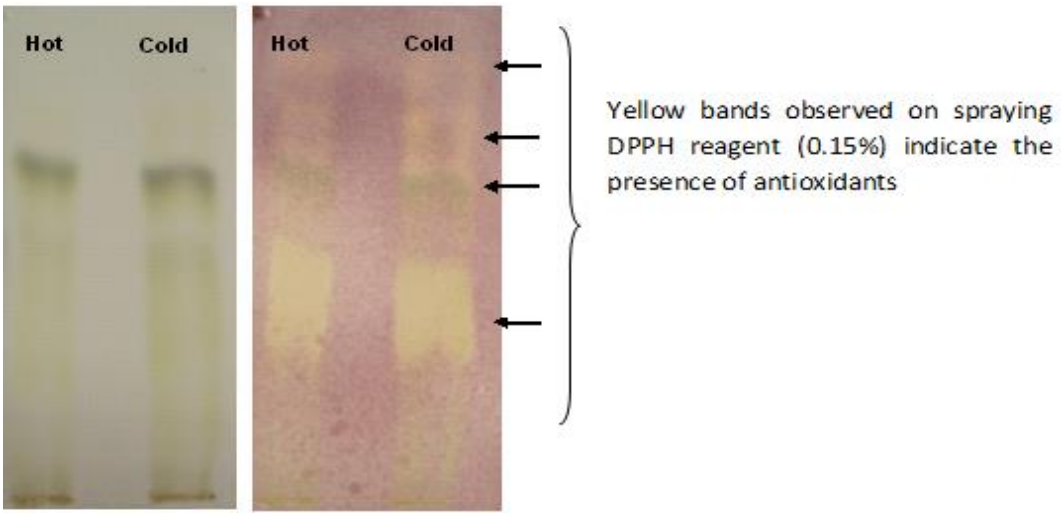

Figure 1: TLC separation of hot and cold chloroform extracts of *Murraya koenigii* (20µg/µl) developed using DPPH spraying reagent (0.15%).

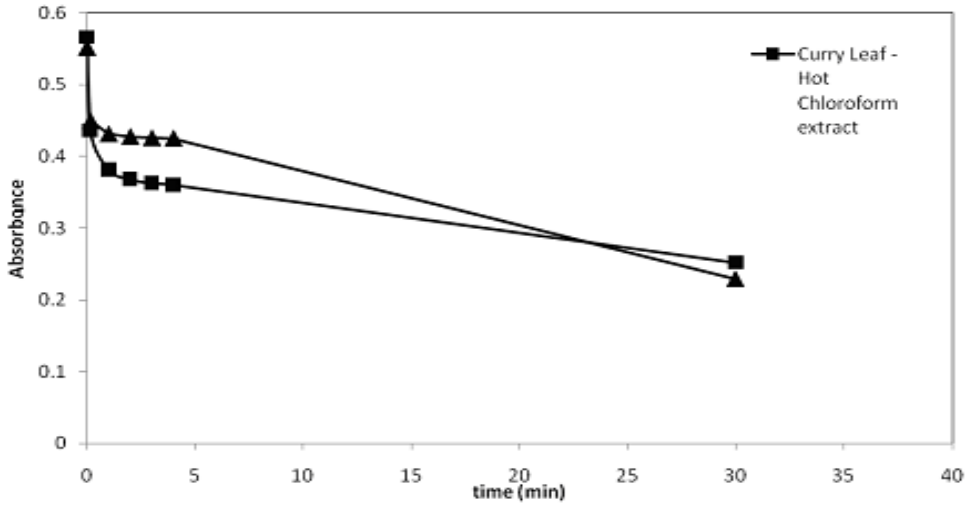

Figure 2: Anti-oxidant activity of hot and cold chloroform extracts of *M.koenigii* (10mg/ml) using 40µM DPPH solution in methanol.Absorbance was recorded at 517 nm. The graph shows the rate of change in the absorbance of DPPH with time in the presence of the plant extracts

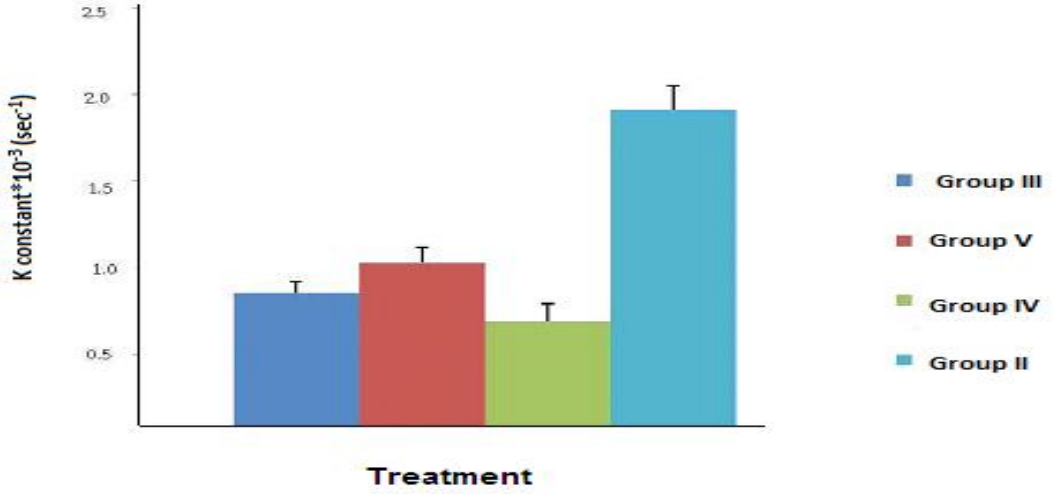

Figure 3: The effect on murine catalase ezyme activity after single dose UVB exposure in the presence and absence of the plant extract. Enzyme activity was determined using the method given by Chance *et al*[27].

1.6W was used for irradiation. The animals were exposed in a black thermocol chamber (external dimensions of 44.0 x 20.5 cms and internal dimensions of 34.5 x 20.5 cms). Two 1 cm^2 exposure sites (one control and another

experimental) were sheared using a sterile hair removing razor, on the dorsal region of the mice, a day prior to the exposure. The study designed was approved by the Institutional Animal Ethics Committee bearing Certificate No. SOS/MSC/IEC-0004.

Animals and Treatments

The animals were randomly divided into five groups each containing six mice. Mice in Group I were exposed to UVB radiation (1.98 J/cm^2) to standardize the minimal erythemal dose. Those in Group II remained unexposed to UVB radiation and served as the control group. Group III mice were unexposed to UVB radiation, however remained in the restrained condition in order to determine the effect of restraining on the enzyme activity. Mice in Group IV were exposed to UVB radiation (3.96 J/cm^2) to study the adverse effects of UVB induced oxidative stress in murine dermis while mice in Group V were exposed to UVB radiation (3.96 J/cm^2) in the presence of the *M.koenigii* cold chloroform extract. The extract was topically applied dissolved in a herbal base in 1:8 ratio. In Groups IV and V, two sites (1 cm^2 each) were sheared onto the dorsal region of the mice one being the area under study and the other control site. In both cases, the control site remained unexposed throughout UVB exposure.

Minimal Erythemal Dose (MED) Determination

Although subjective, erythema is one of the primary indicators of dermal oxidative stress. Minimal erythemal dose (MED) was determined to establish the minimum intensity of UVB radiation required to induce erythema in murine dermis. The UVB intensity required to induce stress was standardized accordingly. The MED was determined to be 1.98 J/cm^2 at a distance of 6.2 cm, on being measured spectrophotometrically (Konica Minolta, Japan). UVB dosage of 2MED (3.96 J/cm^2) was standardized as the dose required to induce dermal oxidative stress.

Preparation of dermal lysate

Mice were humanely sacrificed following single dose of UVB. The sheared dorsal site was immediately excised in cool, sterile conditions to avoid enzyme denaturation. The tissue was homogenized in 100 volumes of ice-cold 10 mM potassium-phosphate buffer (pH 7.4), supplemented with 30 mM KCl using the method proposed by Grankvist *et al*[25]. For the estimation of catalase activity, 100-150 mgs of mice dermal tissue was homogenized in 10 volumes of 10 mM potassium-phosphate buffer (pH-7.4), supplemented with 30 mM KCl and 1% ethanol to avoid the formation of catalase II compound. The homogenates were centrifuged at 12,000 rpm for 15 minutes. The clear supernatant was then stored at -20^{o}C until further analysis[25]. Protein estimation of processed tissue homogenate was carried out by Lowry's method[26].

Enzymatic Quantification of stress enzymes

Determination of catalase and superoxide dismutase enzyme activity.

Catalase (CAT) Activity

Catalase activity was measured as per the method given by Chance *et al*[27]. The reaction mixture contained 0.5 mL of 19.6mM hydrogen peroxide in 10 mM potassium-phosphate buffer. This served as the control sample. Absorbance was recorded at 240 nm using V-550 UV-Vis spectrophotometer (Jasco, Japan). The decomposition of hydrogen peroxide was catalysed by the addition of 50 µL of the tissue homogenate (100 µg/ml) and the decrease in absorbance recorded for 2 minutes. Potassium-phosphate buffer (10 mM) served as the blank. The enzyme activity was expressed in terms of the velocity constant, K (sec^{-1}) of hydrogen peroxide degradation reaction.

Superoxide dismutase (SOD) Activity

Superoxide dismutase activity was assayed by Marklund and Marklund's method[28]. A typical enzyme reaction mixture contained 1.0 mL of 0.4mM pyrogallol in 50 mM Tris-Cl buffer with 1mM EDTA (pH-8.3). Auto-oxidation of pyrogallol was inhibited by adding 150 µL of the processed tissue extract (100 µg/ml) and the inhibition of auto-oxidation recorded spectrophotometrically at 420 nm till 1 min. Consecutive spectroscopic measurement was facilitated by the Time Course Measurement mode in the Spectra Manager Software of the UV-Vis spectrophotometer used. The specific activity of the enzyme was expressed as units/mg protein, where one unit was defined as the amount of SOD sufficient to inhibit the rate of auto-oxidation of pyrogallol by 50%.

Native Gel Electrophoresis of Catalase and Superoxide Dismutase enzymes

Native PAGE was performed using a 7.5% gel and run at 25 mA for 2 hrs at 4^{0}C. Triplicate loading of protein samples (100 µg protein per lane) was carried out to facilitate simultaneous gel staining for total protein, catalase and superoxide dismutase. First one-third of the gel was used for visualizing total protein present in the dermal tissue using Coomassie Blue staining solution, the second half of the gel was used for detecting catalase enzyme[29] while the third part of the gel was used for visualizing superoxide dismutase enzyme[30]. For the detection of catalase and superoxide dismutase, after the electrophoretic run, the gel sections were respectively cut and soaked in their developing solutions. Catalase bands were observed on pretreating the gel with 10mM hydrogen peroxide solution in potassium-phosphate buffer (10mM, pH = 7.0) in dark for 20 mins. followed by a 15 minute incubation. in 1% $K_3Fe(CN)_6$ and $FeCl_3$. Superoxide dismutase enzyme was detected by pre-incubating the gel section in dark for 30 mins in 1M Tris-Cl solution (pH-8.2) containing 10 mg NBT (nitroblue tetrazolium), 75 mg disodium EDTA and 3 mg riboflavin followed by a 15 min. illumination in normal white light.

Statistical Analysis

For parametric variables, analysis of significance of difference between Groups I and II was performed using student's unpaired t-test. All data presented are the mean values of four mice per treatment group combined from two separate experiments. Analysis was performed using Microsoft Excel version 2007.

RESULTS AND DISCUSSION

Anti-Oxidant Content of Plant Extracts

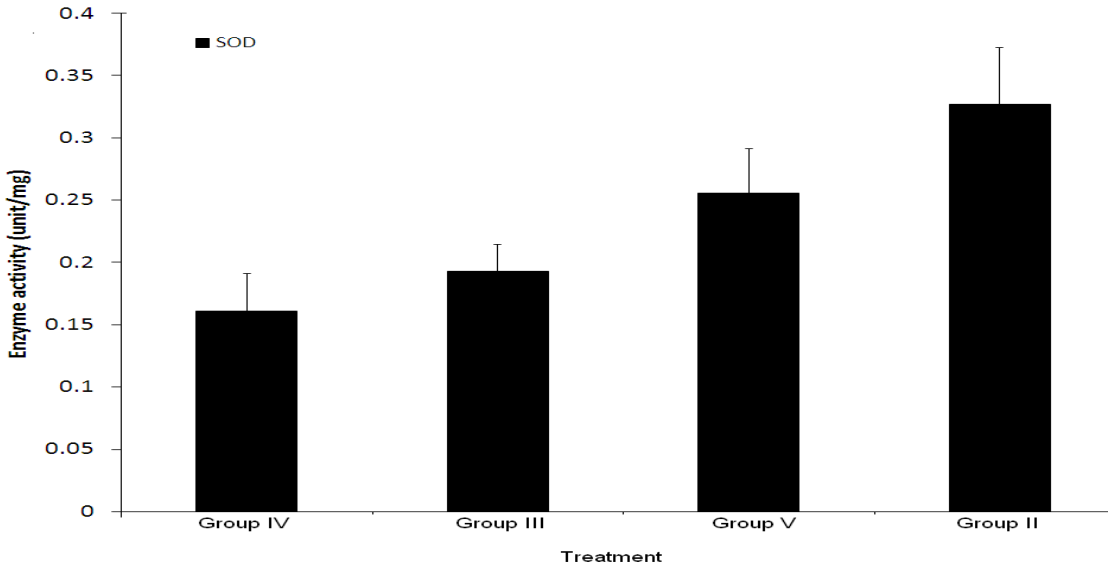

Figure 4: The effect on murine superoxide dismutase enzyme activity after single dose UVB exposure in the presence and absence of the extract. Enzyme activity was determined by Marklund and Marklund's method[28].

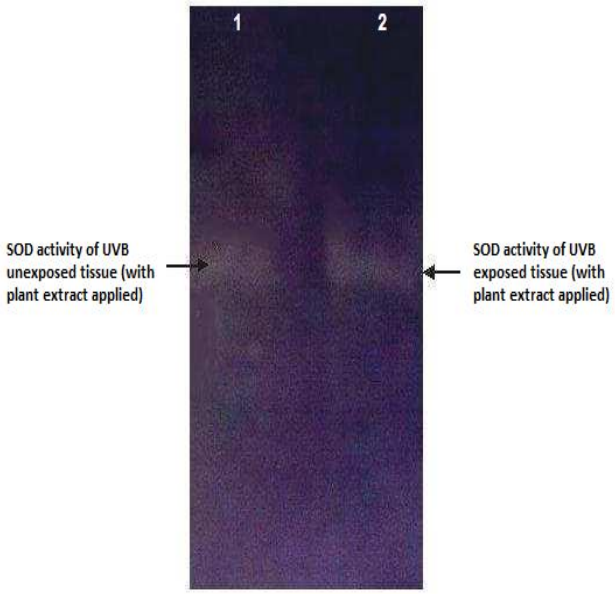

Figure 5: indicates the presence of enzyme superoxide dismutase stained using NBT rolution. The presence of colourless bands against purple background confirmed the presence of superoxide dismutase enzyme in the mice dermal tissue homogenate. Lane 1: Band showing SOD enzyme isolated from unexposed mice tissue homogenate. Lane 2: Band showing SOD enzyme isolated from unexposed mice tissue homogenate. The concentration of protein used was 100µg/ml.

The crude extracts of *M. koenigii* were screened for anti-oxidant activity qualitatively and quantitatively using DPPH reagent as described by Sanchez-Moreno[21]. TLC-DPPH method of determining the radical scavenging activity of the plant extracts qualitatively (Figure 1) showed that the hot and cold chloroform extracts of *M. koenigii* contained 4 major yellow bands indicating the presence of anti-oxidants. Chloroform extracts of Curry leaf obtained by Hot and Cold Extraction methods exhibited an IC_{50} value of 17.46 µg/µL and 16.06 µg/µL respectively in comparison with ascorbic acid used as the standard antioxidant which showed an IC_{50} value of 0.0129 µg/µL. The scavenging activity of the two extracts was observed to last uptil 30 mins., thereby indicating their probable applicability as long-term photo-protectives. Phytochemical extraction using chloroform gave better antioxidant yield than other solvents, probably due to the non-polar nature of the antioxidant compound. According to previous studies carried out by Rao *et al*[18] and Ningappa *et al*[19], acetone extract of curry leaf yielded the highest amount of antioxidant oleoresin while in the case of the latter, the alcohol: water (1:1) extract of curry leaf was observed to have the highest antioxidant and radical scavenging activity However, in the present study chloroform extract of curry leaves was observed to possess the highest antioxidant activity in comparison to hexane, methanol, methanol:water (1:1) and water solvent extracts. Although the cold chloroform extract gave lesser yield (4.21%) as compared to the others, it showed maximum antioxidant activity which was the primary objective in developing an anti-oxidative photo-protective agent effective against UVB. The probable reasons for variable solvent extracts yielding different percentage of antioxidants could be the season, time or geographical region of leaf procurement.

Determination of Med

In comparison to the studies carried out on the effect of UVB exposure on murine dermis by Fuchs *et al*[15], Pence *et al*[13] and Sasaki *et al*[31], the current study focused on determining the extent of dermal stress induced morphologically and physiologically in mice, on spontaneous high dose UVB exposure, at a limited distance separation between the UV source and the mice. The minimal erythemal dose for the present study was observed to be 1.98 J/cm^2 at a distance of 6.2 cms., in the *Swiss albino* mice model. Fuchs *et al*[15] through their

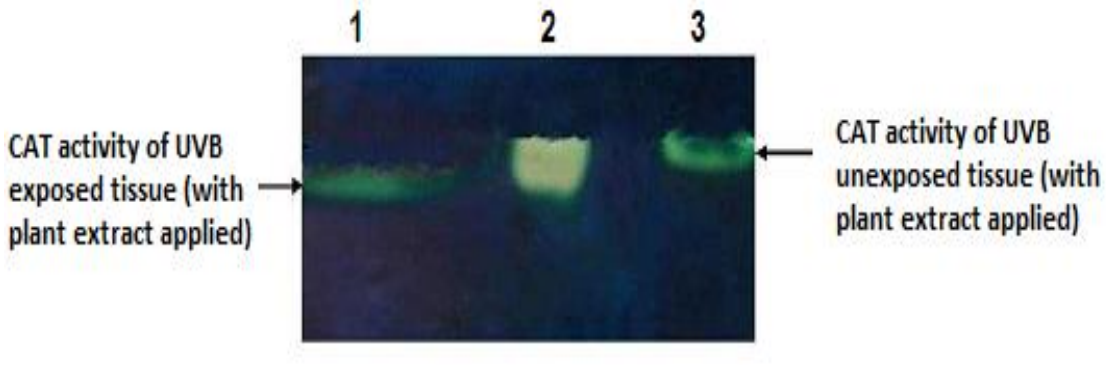

Figure 6: indicates the presence of enzyme catalase stained using Ferricyanide solution. Lane 1: Band showing CAT enzyme isolated from exposed mice tissue homogenate. Lane 2: Band showing commercial CAT enzyme Lane 3: Band showing CAT enzyme isolated from unexposed mice tissue homogenate. The concentration of protein used was 100µg/ml.

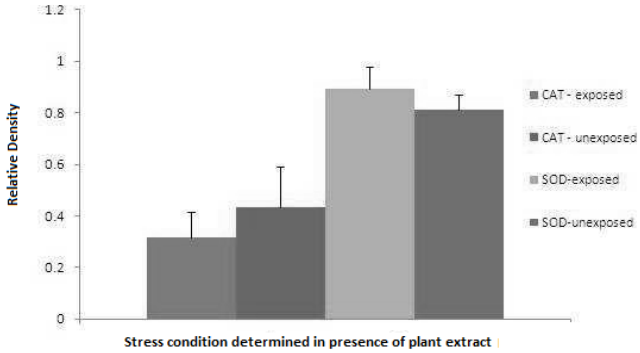

Figure 7: Comparison showing relative band densities of catalase and superoxide dismutase enzymes quantitated from the Native Gel images using ImageJ software.

experiments illustrated the impairment of enzymic and non-enzymic antioxidants in skin on exposure to UVB radiation being as low as 0.36J/cm^2. However, the study was mainly concerned with explaining induction of stress physiologically at the molecular level, irrespective of it being evident morphologically. Hence a low dose UVB exposure was sufficient to induce stress. On similar lines, Sasaki *et al*[31] explained the effect of single dose UVB radiation (1-10 mJ/cm^2) on the activity of superoxide dismutase enzyme. In their study, they used keratinocytes as the *in vivo* model to study stress. Hence, again a small dose of UVB was observed to induce detectable stress. Pence *et al*[13] depicted the effect of single dose UVB radiation on dermal stress enzymes in hairless mice. Dermal UVB dose of 0.09 J/cm^2 was observed to impair the enzymatic activity. However, in their study the mice were placed 30 cm away from the UVB source, thereby requiring a large UVB chamber for exposure which was not feasible in the current experimental scenario. All the previously mentioned findings explained the effect of UVB only at the physiological (enzyme) level. However, they were not able to explain the same morphologically. Determination of stress morphologically (visible erythema development) and physiologically (impairment of enzyme levels) were the key objectives of the current study. Therefore, a UVB dosage of 3.96 J/cm^2 (2MED) was standardized as the UVB dose required to induce spontaneous skin damage and in turn test the plant extract for its photo-protective activity.

Determination of Sod And Cat Activity In Mice Tissue Homogenate

According to previous findings by Pence and Naylor[13] and Sasaki *et al*[31] spontaneous exposure to UVB radiation is responsible for inducing dermal stress. Through their experiments they confirmed the negative effect of single dose UVB exposure on the activity of catalase and superoxide dismutase enzymes. Being in complete congruence with their observations, the data obtained in the current study also indicates decrease in dermal catalase and superoxide dismutase activity on exposure to UVB radiation (3.6 J/cm^2) as compared to the control (Figures 3 and 4). The current study thus also agrees with the hypothesis proposed by Pence and Naylor[13], that UVB treatment is involved in epidermal damage via the generation of free radicals. It is speculated that this damage results in, i) increased consumption of antioxidant enzymes, or ii) UVB induced enzymatic destruction (high UVB instantaneous dosage) at the active Cu or Fe centers via Fenton's reaction. Although the objective of the study was to determine the effect of UVB induced stress on SOD and CAT enzymes, a change in SOD and CAT enzymes was observed on restraining the mice as well. Hence, further analysis of CAT and SOD enzymes was carried out using enzymatic analysis of restrained animals as the baseline to negate any false positive results caused due to restraining. All the data was statistically evaluated using the students T-test ($P<0.05$).

In the presence of the *M.koenigii* cold chloroform extract, superoxide dismutase activity was observed to be significantly higher ($p\leq 0.05$), as compared to mice exposed to UVB treatment with no plant extract applied. Thus, indicating the photo-protective property of the *M.koenigii* plant extract. No significant improvement was observed in the catalase enzyme activity. This might have occurred possibly because of shorter duration of plant extract application. Catalase enzyme is highly sensitive to stress as compared to superoxide dismutase enzyme[32] and therefore probably takes time to regain its lost activity.

Increasing the application time of the plant extract probably might help improve the catalase enzyme activity. Enzyme profiling using Native Gel Electrophoresis further confirmed the adverse effects of stress on the activities of the enzymes catalase and superoxide dismutase. Activity staining of the gel for catalase and superoxide dismutase showed the presence of colourless/pale yellow band on dark blue-green background for the former while the latter appeared as colourless bands on a purple background (Figures 5 and 6). Relative densities of the protein bands were calculated (Figure 7) using the Image J software. On gel quantification, an enhanced level of SOD activity (Figure 6) was observed in the presence of the plant extract. However, no significant improvement was seen in the catalase enzyme activity in the presence of the plant extract. These findings confirmed the results obtained in the biochemical enzyme assay (Figures 3 and 4). Role of phytochemicals as potential photo-protectives has been extensively studied. Some of the work by Wei Huachen *et al*[12], demonstrated the inhibition of non-enzymic oxidative molecules induced on exposure to UVB in the presence of isoflavone genistein extract. Afaq[10] and Vayalil *et al*[11] illustrated an integral role of phytochemicals against UVB induced damage. Significant inhibition in the UVB-mediated induction of cycloooxygenase and ornithine decarboxylase (ODC) enzyme activities following topical application of resveratrol was demonstrated by Afaq *et al*[10]. This was followed by the work done by Vayalil *et al*[11], where they illustrated the preventive role of topically applied green tea polyphenols against UVB induced dermal oxidative damage. In their study, decrease in the level of stress enzymes - catalase, glutathione peroxidase and reduced glutathione (GSH) confirmed the photo-protective role of green tea polyphenols. Although there have been a couple of studies evaluating the photo-protective nature of phytochemicals against UVB by investigating their effect on oxidative stress induced dermally not much has been studied yet. The current investigations thus were carried out with an objective to determine the effect of stress induced as a result of spontaneous exposure to UVB radiation in the presence and absence of the plant extract. The results suggest that acute UVB radiation induces stress marked by decrease in catalase and superoxide dismutase enzyme activity. Significantly enhanced levels of superoxide dismutase activity in the presence of the cold chloroform extract of *M.koenigii* indicates its role as a photoprotective agent, emphasizing the role of antioxidant rich phytochemicals in dermal protection.

CONCLUSION

The present study thus helps explain the relationship between oxidative stress and topical application of antioxidant rich plant extract used as a supplementary replenishment of the depleted antioxidant store in the murine dermis. Improvement in superoxide dismutase activity, post acute UVB exposure, in presence of the topical application of *Murraya koenigii* extract suggest the probable application of the chloroform extract of *M.koenigii* as a photo-protective agent against UVB induced acute oxidative damage. Further, studies are being carried out in assessing the photo-protective effect of chloroform extract of *M.koenigii* applied in various concentrations against varying UVB doses.

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ABBREVIATIONS

UV: Ultra-violet, CAT: catalase, SOD: superoxide dismutase, DPPH: 1,1-diphenyl-2-picrylhydrazyl.

REFERENCES

1. Sinha, R.P., Hader, D.P. UV- induced DNA damage and repair: a review. *Photochem. Photobiol. Sci.* 1, 225–236 (2002)
2. Clydesdale, G.J., Dandie, G. W., Muller, H.K. Ultraviolet Light induced injury: Immunological and inflammatory effects. *Immunol Cell Biol* 79, 547–568 (2001)
3. Matsumura, Y., Ananthaswamy, H.N. Toxic effects of ultra-violet radiation on the skin. *Toxicol Appl Pharm* 195, 298– 308 (2004).
4. Matsumura, Y., Ananthaswamy, H.N. Short- term and long- term cellular and molecular events following UV irradiation of the skin: implications for molecular medicine. *Expert reviews in molecular medicine*. (2002).
5. Gruijl, F.R. Skin Cancer and Solar UV radiation. *Eur J Cancer* 35(14), 2003-2009 (1999).
6. Naylor, M.F., Farmer, K.C. The Case for Sunscreens, A Review of Their Use in Preventing Actinic Damage and Neoplasia. *Arch Dermatol* 133, 1146-1154 (1997).
7. Stewart, M.S., Cameron, G.S., Pence, B.C. Antioxidant Nutrients protect against UVB-induced Oxidative Damage to DNA of Mouse Keratinocytes in Culture. *J Invest Dermatol* 106, 1086-1089 (1996)
8. Fuchs, J. Potentials and Limitations of Natural Antioxidants RRR-alpha- tocopherol, L-ascorbic acid, and beta-carotene in cutaneous photoprotection. *Free Radical Bio Med* 25(7), 848–873 (1998).
9. Svobodova, A., Psotova, J., Walterova, D. Natural Phenolics in the UV- induced skin damage- A Review. *Biomed Papers* 147(2),137-145 (2003)
10. Afaq, F., Adhami, V.M., Ahmad, N. Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. *Toxicol Appl Pharm 186, 28–37* (2002).
11. Katiyar, S.K., Bergamo, B.M., Vyalil, P.K., Elmets, C.A. Green tea polyphenols: DNA photodamage and

photoimmunology. *J Photochem Photobio B: Biology* 65, 109–114 (2001).
12. Wei, H., Zhang, X., Wang, Y., Lebwohl, M. Inhibition of ultraviolet light-induced oxidative events in the skin and internal organs of hairless mice by isoflavone genistein. *Cancer Letters* 185,21–29 (2002).
13. Pence, B.C., Naylor, M.F. Effects of Single-Dose Ultraviolet Radiation on Skin superoxide Dismutase, Catalase, and Xanthine Oxidase in Hairless Mice. *J Invest Dermatol* 95(2), 213-216 (1990)
14. Shindo Y., Witt, E., Packer, L. Antioxidant Defence Mechanisms in Murine Epidermis and Dermis and Their Responses to Ultraviolet Light, *J Invest Dermatol* 100,260-265 (1993)
15. Fuchs, J., Huflejt, M.E., Rothfuss, L.M., Wilson, D.S. *et al*. Impairment of enzymic and non-enzymic antioxidants in skin by UVB irradiation. *J Invest Dermatol* 93,769-773 (1989)
16. Fuchs, J., Huflejt, M.E., Rothfuss, L.M., Wilson, D.S. *et al.* Acute effects of near ultraviolet and visible light on the cutaneous antioxidant defense systems. *Photochem Photobiol* 50,739-744.
17. Mishra, J., Yousuf, A., Singh, R.D., Aradhana. Phytochemical Investigation and in-vitro antioxidant potential of leaves of *Murraya koenigii*. *IJIB* 7(3),171 (2009).
18. Rao, L.J.M., Ramalakshmi., Borse, B.B., Raghavan, B. Antioxidant and radical-scavenging carbazole alkaloids from the oleoresin of curry leaf (*Murraya koenigii* Spreng.). *Food Chemistry* 100,742–747 (2007)
19. Ningappa, *M.B., Dinesha, R., Srinivas, L.* Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (Murraya koenigii L.) extracts. *Food Chemistry*, 106,720–728 (2008)
20. Kokate, C.K., Purohit, A. P., Gokhale, S. B. Pharmaconosy, Nirali Prakashan Ltd., Pune (2007)
21. Sánchez-Moreno C., Larrauri, J.A., Saura-Calixto, F. *A* procedure to measure the antiradical efficiency of polyphenols. *J Sci Food Agric* 76, *270–276* (1998)
22. Saha, M.R., Hasan, S.M.R., Hossain, M.M., Alam, M.S., Alam, M.A., Mazumder, M.E.H. *In vitro* Free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi Linn. Bangl J Vet* 6(2),197–202 (2008)
23. Arokiyaraj,S., Martin,S., Perinbam,K., Arockianathan P.M., Beatrice,V. Free radical scavenging activity and HPTLC finger print of *Pterocarpus santalinus* L. – an *in vitro* study, Indian Journal of Science and Technology, 1(7), 1-3 (2008)
24. Policegoudra, R.S., Abiraj, K., Gowda, D.C., Aradhya, S.M. Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome. *J Chromatogr B*, 852, *40–48 (2007).*
25. Grankvist, K., Marklund, S., Täljedal I-B. Cu-Zn superoxide dismutase, Mn- superoxide dismutase, catalase, glutathione peroxidase in pancreatic islets and other tissues in the mouse, *Biochem J* 199, 393-398 (1981).
26. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. Protein Measurement with the Folin Phenol Reagent, *J Biol Chem* 193, 265–275 (1951).
27. Chance, B., Herbert, D. The enzymes substrate compounds of bacterial catalase and peroxides. *Biochem J* 46(4), 402–414 (1950)
28. Marklund, S., Marklund, G. Involvement of Superoxide *Anion Radical* in the Autooxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. *Eur J Biochem* 47, *469-474* (1974*).*
29. Woodbury, W., Spencer, A.K. and Stahman, M.A. An improved procedure using ferricyanide for detecting catalase isozymes. *Anal Biochem* 44:301–305 (1971).
30. Greneche, M., Lallemand, J., Michaud, O. Comparison of different enzyme loci as a means of distinguishing ryegrass varieties by electrophoresis. *Seed Sci. Technol.*, 19:147–158 (1991).
31. Pigeolet, E., Corbisier,P., Houbion,A., Lambert, D., Michiels,C., Raes, M., Zachary,M.D., and Remacle, J. Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mecha- nisms of Ageing and Development*, 51:283 – 297 (1990).
32. Pigeolet, E., Corbisier,P., Houbion,A., Lambert, D., Michiels,C., Raes, M., Zachary,M.D., and Remacle, J. Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mechanisms of Ageing and Development*, 51:283 – 297 (1990).