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Inhibition of UV-induced oxidative stress in Bovine Lens by using an ethanolic bark extract of Mitrgyana pervifolia

Amala Vallabhaneni, Bhaskar Nagilla, Meenakshi*

(Department of Zoology, University College of Science, Osmania University, Hyderabad)

Abstract:
UV-B radiation may be a significant risk factor in cataract development. After being exposed to UV-B radiation, cells exhibit abnormalities in the repair of DNA damage, resulting in alterations in the amounts of specific proteins. Mitrgyana *pervifolia* has been used in Ayurveda for its variety of medical properties. The aim of this study is to evaluate the impact of an ethanolic extract of Mitgyana pervifolia on UV-induced oxidative stress in bovine lenses as well as to provide details on the lens's antioxidant defenses and how they are controlled. Protein profiling was analyzed and quantitated with SDS-PAGE, Oxidative stress mediators like Catalase (CAT), Glutathione reduced (GSH) and Superoxide Dismutase (SOD) were estimated. The data showed that between the three experimental samples, the intensities of several protein bands varied. Mitrgyana-treated lenses displayed lengthier protein patterns and more pronounced banding. While lenses exposed to UV light exhibited lower levels of proteins, catalase, GSH, and SOD, lenses treated with Mitgyana demonstrated a recovery of antioxidant status. The treatment with Mitgyana decrease in the oxidative stress in the Bovine lenses.

Keyswords: UV radiation, Mitrgyana pervifolia, SDS-PAGE, Catalase, Glutathione reduced, Superoxide Dimutase.

Introduction:

A cow's eye is quite similar to a human's eye. The lens of the eye appears to be a perfect environment for the production of amyloid fibrils. The transparent and very high protein content of the vertebral eye lens is two noteworthy features. The water soluble lens proteins have been the subject of extensive research for around nine decades. But recently, the attention has switched to the lens' structural proteins. The lens is an excellent spot to start when trying to isolate different enzymes. Different species have recruited a number of proteins to operate as structural proteins in the lens (Wisow & Piatigorsky, 1987). A large concentration is required in order to provide an adequate refractive index; hence a high solubility is one requirement for such a protein. Proteins play an important part in the lens's transparency to visible light. In favour of an alternative technique that relies on the long-term stability of the proteins, all organelles have been scattered inside the lens centre. Oxidative stress is mediated by ultraviolet radiation. Recently, an increase in the danger of UV rays to the eye has been related to a decrease in the stratospheric zone. Health hazards connected with ozone depletion are induced by changes in UV radiation in the environment as well as greater penetration of shorter wavelength rays. (Between 280nm to 320nm) Zigman 1993, Tenkate 1999, Kabuyama et al., 2002).

The most vulnerable element of the eye is the lens, which is vulnerable to UV damage. Chronic eye conditions such as cataract, squamour cell carcinoma, ocular melanoma, and a variety of corneal and conjunctival illnesses (Ex.pterugium & Pinguecula) tend to worsen with Ozone depletion (De Gruil 2000, Berwick 2000.

Mitragyna parvifolia, a Rubiaceae family member, is a medically and commercially significant deciduous tree. The stem of Mitragyna parvifolia (Roxb.) measures around 2 mm thick. The bark can be divided into an inner bark (secondary phloem) and an outer bark (periderm). Phloem that has collapsed on both the outside and inside of the inner bark. According to Jain *et al.* (2009) methanolic extract of Mitragyna parvifolia (MEMP) leaves were found antiarthritic and antipyretic potential in rodent. Gupta *et al.* (2009) investigated both anti-inflammatory and antinociceptive activity of the ethanolic extract of dried leaves of *Mitragyna parvifolia* (MPEE), using the Carrageenan-induced paw edema and Tail-flick method in rodent. Recent findings by Ghatak *et al.* (2014**)** showed that five different extract of dried bark and leaves of Mitragyna parvifolia in distilled water, methanol, acetone, ethyl acetate and hexane were evaluated for antioxidant potential, lipid peroxidation and antiproliferative effect on HeLacell lines. Antioxidant potential was investigated using DPPH radical scavenging activity (Gajendra Pratap Choudhary and Ashutosh 2016).

Material and Methods:

Preparation of plant extract: The **Mitrgyana *pervifolia*** plant was gathered in Telangana's Adilabad district's Jannaram forest. The Department of Botany verified the plant's authenticity. The Bark was put to use in experiments. It was cleaned with running water, air dried in the shade, then powdered. The powder was extracted with ethanol using the Soxhlet apparatus, and the crude extract was used for experimentation.

UV cross-linkng: Mature male bovine lens collected from the abattoir and transported to the lab on dry ice. Each eye had a posterior sclerectomy, the zonules were incised with a Sharpe sterile blade, and the lens was gently spooned out and placed on the culture dish before being rinsed with sterile ice cold PBS buffer pH 6.8. Each culture dish was outfitted with TC199 culture medium and a mixture of 100 units of penicillin and 100ug of streptomycin was added to each plate.

And the lens was grouped in three groups:

Group-1: Control lens

Group-2: Control lens + UV exposure

Group-3: Control lens + UV exposure + Plant extract

UV exposure was achieved by putting the lens in a UV cross linker (Amarsham Company) for three hours.

Biochemical test:

Proteins were estimated by Biuret method (Alois Nowotny 1979)

Antioxidant Assays:

Catalase was estimated by Aebi, 1984 method, by monitoring the disappearance of Hydrogen peroxide (H_2O_2).

Glutathione reduced (GSH) was estimated according to Hissin & Hilf, 1976.

Superoxide Dismutase (SOD) was estimated according to Christine J. Weydert and Joseph J, 2010.

Lens protein profile by SDS – PAGE: Laemmli (1970).

Chemicals required :

1. Preparation of stock solution and buffers:

30% acrylamide

a)Acrylamide : 29.2gm

b) N,N-methelyne –bis –acrylamide:0.8gm. Added water, dissolved and made upto 100ml and filtered with Whatman filter paper.

2. Separating gel buffer:

a)Tris-HCL: (1.5M , pH 8.8)

18.171g of Tris was dissolved in 60ml of water and adjusted the pH 8.8 with 0.1N HCL and finally made up to 100ml with water.

3.Stacking gel buffer :

a) Tris –HCL: 1M, (pH 6.8)

6.057g of Tris was dissolved in 60ml water and adjusted the pH to 6.8 with 0.1N HCL And made upto 100ml with water.

4.10% SDS solution:

1g of SDS in 10 ml of distilled water.

5.N,N,N'N'-Tetra methylene diamine (TEMED).

6.10% Ammonium per sulphate (APS):1g of APS in 10 ml of distilled water .

7. Electrophoresis Buffer:

a) Tris : 25m M , p H 8.3

b) Glycine : 250m M ,p H 8.3

c)SDS 0.1% :dissolved in minimum amount of water(500ml)and then added SDS. Allowed to settle and dissolved. This was finally made upto 2.5liters.

8.Sample buffer 4X : 5ml

a) Tris(1M,p H 6.8):2.1ml

b) 2% SDS : 100mg

c) Glycerol (100%):1ml

d) b-mercaptoethanol:0.5ml

e) Bromophenol blue :2.5mg

f) Distilled water:0.4ml

9.Staining solution (100ml)

a) Alcohol(Methanol):40%
b) Glacial acetic acid:10%
c) Commassie Brilliant Blue (CBB –R-250):259mg
d) Distilled water: 50%

10.Destaining solution (100ml)

a) Alcohol(Methanol):50%
b) Glacial acetic acid :10%
c)Distilled water :40%

PROCEDURE:

Preparation of gel:

The glass plates were washed in warm detergent solution, rinsed subsequently in tap water, deionised water and ethanol and dried. The unnotched outer plates were laid on the table and vaseline was coated. Spacer strips were arranged approximately at the sides and bottom of the plates. The notched inner plates were laid in position, resting on the spacer strips and the arrangement was mounted vertically. Sealing was done properly to avoid leakage. The volume of the gel solution required for making separating gel was calculated as follows:

For 10 % Resolving gel :

H_2O:7.9ml, 30% Acrylamide: 6.7ml, 1.5M Tris (p H 8.8):5ml, 10%SDS: 0.2ml, 10% APS: 0.2ml, TEMED: 0.008.

APS and TEMED were added just prior to the pouring of gel. The solution was mixed well and poured into the space between the two plates leaving an inch of the upper space unfilled. Water was carefully laid over the surface of the poured gel mixture to avoid air contact, which reduces the polymeraization reaction. The gel mixture was allowed to polymerise, undisturbed at room temperature at 60 min. In the mean time gel mixture for stacking gel was prepared.

After the separating gel was polymerized the over laid water was removed carefully with filter paper and an appropriate comb was inserted between the plates. 0.1ml of 10 % APS and 0.01 of 10% TEMED were added to the stacking gel mixture.it was mixed well and poured immediately over the separating gel. The stacking gel was allowed to polymerise.

For 10% Stacking gel:

H_2O:6.8ml, 30% Acrylamide: 1.7ml, 1M Tris HCL, pH 6:1.25ml, 10%SDS: 0.1ml
10%APS: 0.1ml, TEMED: 0.01ml.

Preparation of protein sample:

The required volume of sample buffer was added to protein samples and they were loaded (the final concentration of sample buffer in the prepared sample should come to 1X .If the protein was dried suspended it in 1X buffer). The samples were incubated for 2 minutes in a boiling

water bath prior to loading. When the polymerization was completed the comb was removed and the lower spacer strip qas carefully removed. The Vaseline from the bottom was removed with a piece of tissue paper. The gel was attached to the electrophoresis tank using appropriate clips /clamps. The lower reservoir was filled with 1X electrophoresis buffer, using a bent Pasteur pipette or syringe needle to remove any air bubble trapped beneath the
bottom of the gel. The protein samples were loaded using a micropipette and the wells were completely and carefully filled with 1X electrophoresis buffer. The upper reservoir was also carefully filled with 1X electrophoresis buffer. The electrodes were connected to a powerpack.
The gel was run at constant current for 4 to 6 hrs at room temperature. Electrophoretic mobility of the samples was determined by Bromophenol Blue Front. At the end of the run the power pack was switched off. The gel and plates were laid flat on the table and a corner of the upper glass plate was lifted up and the gel was carefully removed.

Staining of the gel :

After the completion of the electrophoresis, the gel was fixed with 10%Trichloroacetic acid for 5 minutes and stained with CBB. The CBB staining solution was prepared using methanol,acetic acid and double distilled water in the ratio of 4:1:5 ratio and 0.25g of CBB was added and the gel was stained over night.

Destaining of the gel:

The destining of CBB stained gel was done by using methanol, acetic acid and double distilled water in the ratio of 5:1:4 ratios till the appearance of clear bands on the gel.

Statistical analysis

All results were present as mean± SD. Statistical analysis of all the data obtained was evaluated using One way ANOVA test, t-Test: Two-Sample Assuming Unequal Variances.

Results:

Protein quantification is considered to be the ultimate change in affecting the tissue functions and therefore, we analyzed the total protein content in all groups and we have observed a considerable decrease in total protein was observed in group II compared to group I and supplementation of plant extract improved total protein levels in group III (Fig.1).

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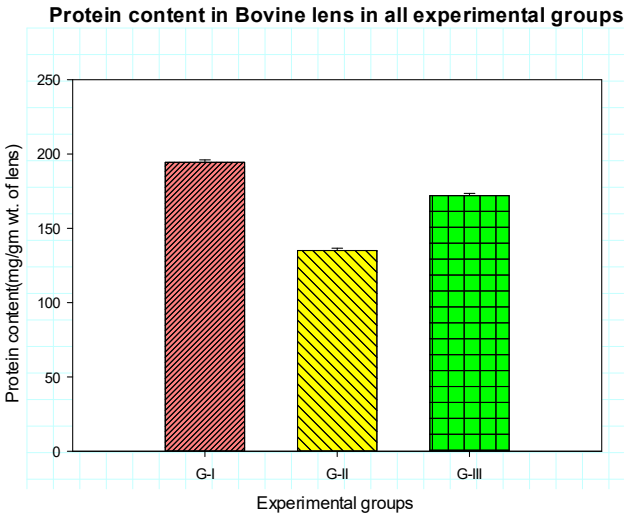

Fig.1: Effect of Mitrgyana *pervifolia* on protein quantity of all experimental groups.
(Values represented as Mean± SD, protein content expressed as in mg/gm wt., of lens, all values are significant with $p<0.05$).

Catalase activity:

There was a rise in catalase activity due to high UV related oxidative stress. However, activities were normalized due to the addition of plant extract in group III (Fig.2) Catalase is a primary defensive enzyme against H_2O_2 in many tissues. We found that the increase in Catalase activity in the UV induced oxidative stress lens was significantly decreased by the treatment of plant extract, probably due to the elevated levels of GSH-Px, which can compensate for the activity of Catalase when the hydrogen peroxide levels were low.

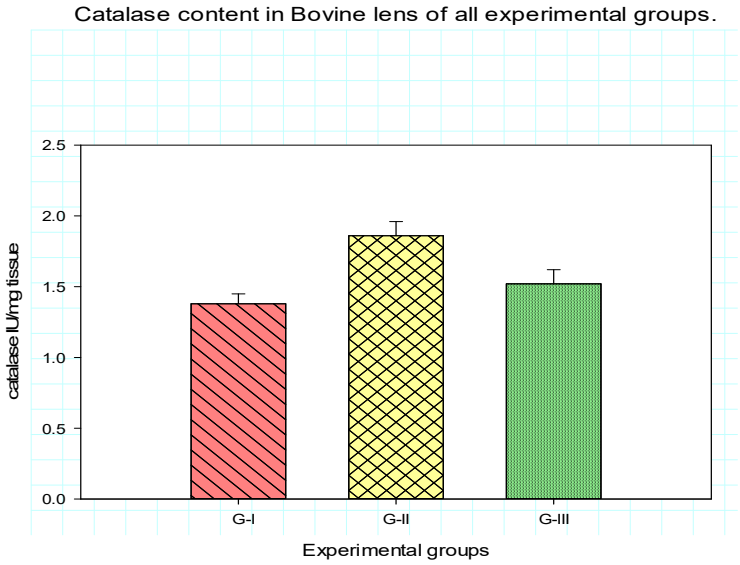

Fig.2: Effect of Mitrgyana *pervifolia* on catalase of all experimental groups.
(Values represented as Mean± SD, Catalase expressed as in IU/mg tissue, all values are significant with $p<0.05$).

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GSH: GSH is the first line of defense against oxidative stress; therefore the level of decreased GSH was assessed in all experimental groups, as shown in Fig.3. GSH levels were lower in group 2 (UV-exposed lenses) compared to control lenses. Group-III (Bark extract) demonstrated significant GSH recovery in the lens.

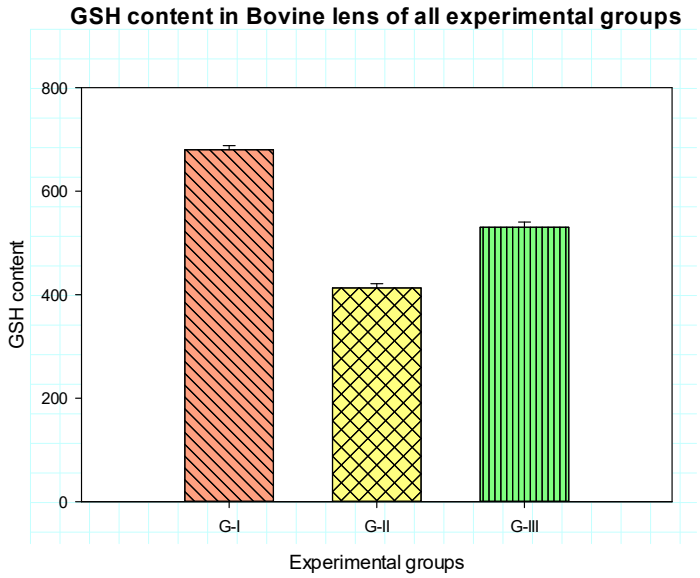

Fig.3: Effect of Mitrgyana *pervifolia* on GSH of all experimental groups.
(Values represented as Mean± SD, GSH expressed in nμ/gm tissue, all values are significant with $p<0.05$).

SOD:
Fig.4 depicts the results of SOD activity in lenses of all experimental groups. Control group-I showed normal activity of SOD. But experimental (UV exposed) group-II showed significantly lower levels of SOD activity when compared with control (G-I). In comparison to the experimental group II, the treated group III demonstrated a considerable recovery of SOD activity.

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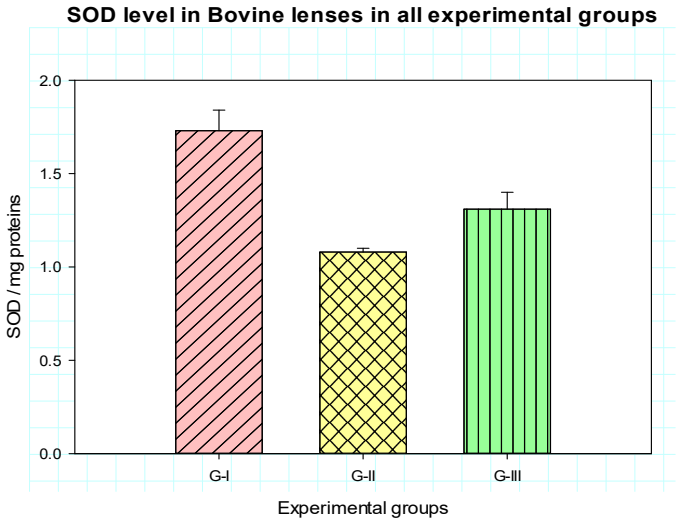
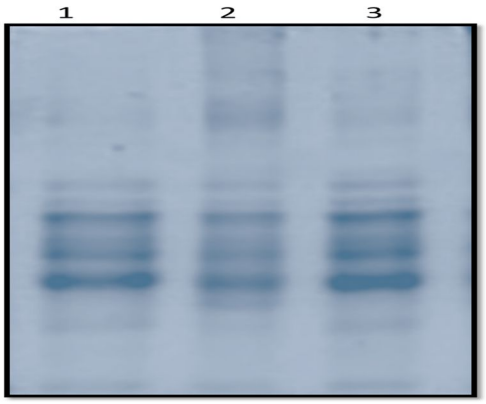


Fig.4: Effect of Mitrgyana *pervifolia* on GSH of all experimental groups.

(Values represented as Mean± SD, SOD expressed as in U/mg proteins, all values are significant with $p<0.05$).

Fig.5: Gel picture



Lane1: Control lens
Lane 2: Control +UV EXP.+ Plant Ext.
Lane 3: Control +UV EXP

Discussion:

Different components of Mitrgyana *pervifolia* have been examined for their therapeutic potential. Our data show the protective effects of an ethanolic bark extract against UV-induced lens oxidation. The open E ring alkaloids isorhychophylline and rhynchophylline are among the phytochemicals found in the bark of Mitrgyana (Shellard EJ & Houghton PJ in 1971). The anxiolytic efficacy of a methanolic extract of Mitragyana stem barks with a high proportion of alkaloid has been studied in mice (Badgujar Vishal B and Surana Sanjay J, 2009). In addition to its anthelmintic properties **(Badgujar Vishal B and Surana Sanjay J 2010)** the stem bark of Mitragyna parvifolia has been shown to be effective against two yeasts (Saccharomyces cereviseae and Candida albicans) and several human pathogen microbial strains, including Escherichia coli and Pseudomonas aeruginosa (Kumar and Shreya, 2011). Mitragyana barks have high antioxidant qualities in addition to several significant medicinal effects (Ghatak et al., 2014).

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The lens's exposure to UV light is thought to be a major cause of cataract formation in vertebrates (Vrensen, G.F., 1994). The UV radiation induction method was utilized to investigate the role of Mitragyana bark in avoiding lenticular damage. The efficacy of UV radiation in lenticular damage is due to an imbalance between antioxidants and oxidative stress, as demonstrated by catalase in this study. UV radiation exposure caused a significant decrease in SOD and, to a lower extent, GSH levels as antioxidant enzymes, as well as a decrease in catalase levels as a sign of the degree of lens damage. UV light, according to Newsome et al., 1994, is a significant source of free radical damage in tissues, particularly the cornea and lens. According to Newsome et al., 1994, UV light is a very important source of free radical damage in tissues especially cornea and lens. In terms of the mechanism of UV radiation lens cell damage, absorbed UV-photons excite lens molecules, resulting in free radicals leading to oxidative stress on the lens. This causes DNA, protein, and lipid damage. We observed a decrease in protein content in UV-induced lenses in our study, yet there was a recovery in protein content in Mitragyana-treated groups. DNA damage brought on by UV radiation can alter lens cell differentiation and protein synthesis (Michael *et al.,* 1998), which may be the reason for decreased protein content in UV, induced lenses. Perhaps the antioxidant nature of Mitragyana bark might have prevented DNA damage, allowing the proteins in the lenses to remain at their optimum. According to Cejkova *et al*., 2000 SOD and Catalase have secondary role in antioxidant defence in lens. But when an episode of oxidative stress occurs, enzyme activity begins to decline, first CAT activity and then SOD activity, increasing the amount of H_2O_2 and further escalating the lenses damage. In UV exposed both the levels of SOD and CAT activity were dramatically reduced. H_2O_2 production is one explanation for the decline in SOD and fluctuations in CAT activity, offering a mechanism for balance.

GSH is a significant defensive mechanism against photo-oxidation in the lens and aqueous humour (Taylor et al., 1995). GSH is abundant in lenses; however, its concentration declines as a result of UV radiation (Mody *et al.,* 1995) and cataract cases (Sasaki *et al.,* 1995). The level of GSH appears to be the most important indicator of oxidative damage. Our study shows a decrease in GSH activity in UV exposed lenses, showing UV-induced oxidative damage that may be directly related to an increase in H2O generation. GSH content recovered in Mitragyana-exposed lenses, which could be attributed to regulated H2O generation.

UV-induced oxidative stress is related with a decrease in the cell's overall reducing power, as demonstrated by decreased GSH, which plays a critical role in coordinating the body's antioxidant defence activities. GSH scavenges hydrogen peroxide via GSH-Px-catalyzed processes. When the level of H2O2 in the tissues is low, GSH-Px is known to be more effective than SOD and CAT.

A useful method for characterising proteins is gel electrophoresis. Electrophoresis is a helpful and important technology for identifying protein profile in various tissues. All three groups in the present investigation displayed diverse protein banding patterns. High molecular weight proteins

are represented by the dark bands that are visible in all groups; low molecular weight proteins are represented by lighter bands that have migrated further which are not properly resolved. Although the study's findings are not particularly evident, each group's protein profile is very constant across all groups, which is consistent with previous studies (Patwardhan V & Modak S P, 1992).

To conclude this observation prompted the current investigation to assess the effectiveness of medicinal plant extracts in preventing or inhibiting/quenching the production of free radicals and thiol groups and restoring the lens proteins structure and transparency. Exposure of lenses to UV radiation may be a risk factor for the onset of oxidative stress-related lens damage. Mitragyana, a powerful free radical scavenger and antioxidant, may protect rat lens tissues from oxidative damage, minimising disruption of the oxidant/ antioxidant equilibrium in the lens tissue and so reducing organ dysfunction.

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