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ROLE OF PROLINE AND TYROSINE IN HYDROGEN PEROXIDE INDUCED CATARACT

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Abstract

Cataract is an opacification of the lens of eye. Causes of cataract formation are ageing, generation of free radicals, diabetes etc. Antioxidants & antioxidant enzymes protect the eyes by reducing free radical damage.

Scope:- Formulation of eye drops with Proline and Tyrosine and its addition to eyes may delay the process of cataractogenesis..

Material and Methods: Total 120 fresh goat lenses were used for the study. "Lens organ culture technique" was used. Lenses were incubated in in TC-199 culture media for72 hrs. Antibiotics and antifungal agents were added to avoid bacterial and fungal infection. Lenses were divided into following groups,

Group 1(control,30) = TC-199+ lens
Group 2 (Expt- H_2O_2,) = TC-199+ lens +H_2O_2 (30)
= TC-199+ lens+ H_2O_2+Proline (30)
= TC-199+ lens+ H_2O_2+Tyrosine (30)

Expriment was terminated at 72 hrs and Lenses homogenized and supernatant was used for spectrophotometric measurement of total soluble Lens proteins, MDA, Superoxide dismutase, Catalase,Glutathione peroxidase & Glutathione reductase.

Findings: Addition of Proline &Tyrosine in culture media Showed statistically significant decrease in MDA levels(nmol/gmwt)8.97$\pm$2.81, 10.41$\pm$3.55 ($P<0.0001$) And statistically significant increase in Specific activity of antioxidant enzymes(Units/mg protein) like Proline 0.87$\pm$ 0.45, 0.78$\pm$ 0.46, 27.58$\pm$ 15.64, 25.47$\pm$ 15.59 & for Tyrosine were 0.84$\pm$ 0.26, 0.98$\pm$ 0.60, 32.29$\pm$ 18.44, 27.20$\pm$ 13.0($P<0.0001$, $P<0.01$).

Conclusions: Study shows that addition of Proline &Tyrosine in culture media delays the progression of cataract by minimizing free radical formation.

Keywords: MDA – Malondialdehyde, SOD- Superoxide Dismutase, G-Px-Glutathione Peroxidase, H_2O_2- Hydrogen peroxide, GSH- Reduced glutathione, GSSG- Oxidized glutathione.

Introduction

Cataract is one of the leading causes of visual disability leading to blindness in the elderly population. It is defined as any opacity in the lens or its capsule whether developmental or acquired is called cataract (1). Causes of cataract formation are ageing, generation of free radicals, diabetes etc.

Cells have their own supply of endogenous antioxidants for repair of oxidative damage, and depletion of these antioxidants could be the cause for oxidative tissue damage (2).

Superoxide radicals are dismutated by superoxide dismutase into hydrogen peroxide. This hydrogen peroxide is metabolized by catalase into water but this reaction requires reduced glutathione to metabolize hydrogen peroxide. Thus catalase and glutathione peroxidase detoxify $H_2O_2$with the help of reduced glutathione into oxidized glutathione and water. There is need to maintain reduced form of glutathione. So Oxidized form of glutathione is converted back into reduced form with the help of glutathione reductase and NADPH.

Fig1.Redox cycle involving antioxidant enzymes & their role.

Therefore the following objectives were designed.

1. To study the process of cataractogenesis, by using Hydrogen Peroxide induced experimental cataract by using "Lens organ culture technique".
2. To estimate MDA level as an index of Lipid peroxidation.
3. To estimate the conc. of lens total soluble proteins to study the biochemical events in cataractogenesis.
4. To study the effect of Proline & Tyrosine on activity of antioxidant enzymes (SOD, Catalase, GSH-Px, GSH-Rx).

Materials and Methods

Hydrogen Peroxide induced cataract” has been chosen as a model for present study. Goat lenses were used for the development of cataract. Eye balls were obtained from slaughter house and transported to the laboratory in ice- box. Lenses were removed from eyeballs by intracapsular lens extraction method & then were weighed separately.
Total 120 goat lenses were used for the study. These lenses were divided under the following groups…

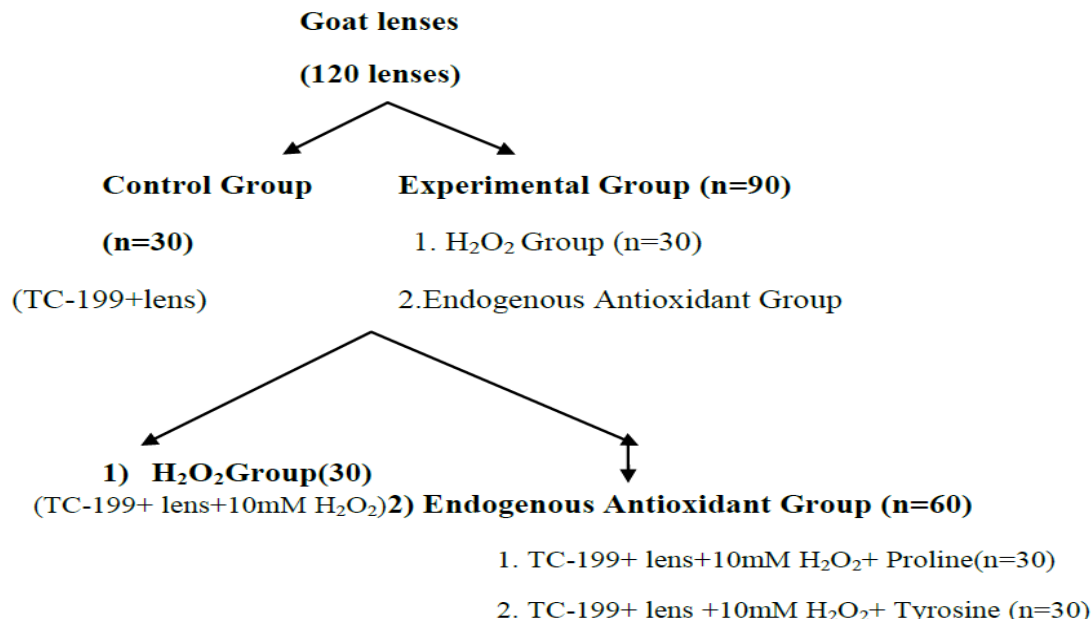

Fig No. 1 Division of lenses into different groups.

Lenses were incubated in TC-199 culture media for 72 hrs. using “Lens Organ culture technique”[(3)]. Penicillin and mycostatin were added in media to prevent bacterial and fungal infection. Lens transferancy was measured by observing number of squares seen through the lens & that was also observed for development of cataract, and noted visually &recorded photographically.

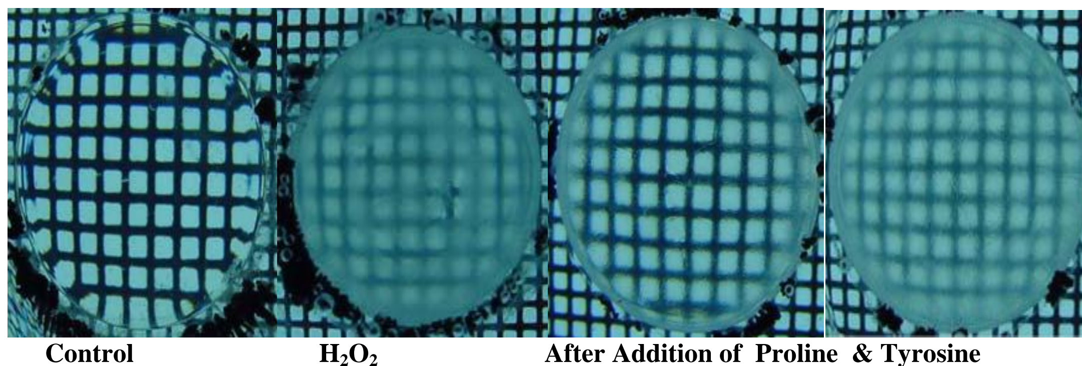

Fig No.2 Lens transferancy was measured by observing number of squares.

Homogenate of cultured lens was prepared in 0.1M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 x g for 30 min at – 4^0C. The supernatant was collected and stored at –20^0C until further use. Lens homogenate of each group was subjected to the following estimations,

1. Total soluble proteins (Lowry’s method,1955)
2. MDA levels (Kei Satoh,1978)
3. Superoxide dismutase (Marklund&Marklund,1974)
4. Catalase (Aebi,1974)
5. Glutathione Peroxidase, by Randoxkit(Paglia&valentine, 1967)
6. Glutathione Reductase, By Randox kit (Goldberg & Spooner, 1975)

Results

Table no. 1 shows statistically significant increase in wet weight (740 $\pm$ 41.0 mg) and MDA levels (19.70 $\pm$ 4.14) in cataractous lens group as compared to control group (660 $\pm$32.8 , 222.53 $\pm$ 48.61) and statistically significant decrease (193$\pm$60.01mg/lens) in total soluble proteins in cataractous lens group compared to control group(222.53$\pm$48.61 mg/lens). We also found statistically significant decrease in specific activity of antioxidant enzymes (0.35 $\pm$0.18, 0.63 $\pm$0.35, 21.8$\pm$12.6, 16.43 $\pm$4.10 in U/mg protein) in cataractous lens group compared to control group (0.98 $\pm$0.26, 0.95 $\pm$0.68, 43.7$\pm$15.7, 30.14$\pm$ 15.54 in U/ mg protein) .

Table 1: Comparison between control and H_2O_2 group and P value.

Group	Wet Weight mg Mean ±S.D	Total soluble lens protein mg/lens Mean ± S.D	MDA nmol/ gmwt Mean ± S.D	SOD Units/mg protein Mean ± S.D	Catalase Units/mg protein Mean ±S.D	GSH-Px Units/mg protein Mean ± S.D	GSH-Rx Units/mg protein Mean ± S.D
Control group	660 ± 32.8	222.53 ± 48.61	9.99 ± 4.00	0.98 ±0.26	0.95 ±0.68	43.7±15.7	30.14± 15.54
H_2O_2 group 10mM	740 ± 41.0 **P<0.01**	193 ± 60.01 **P<0.05**	19.70 ± 4.14 **P<0.001**	0.35 ±0.18 **P<0.001**	0.63 ±0.35 **P<0.05**	21.8±12.6 **P<0.01**	16.43 ±4.10 **P<0.001**

Table 2: Comparison of H_2O_2 group with endogenous antioxidant group (Proline and Tyrosine).

Group	Wet Weight mg Mean ±S.D	Total soluble protein (mg/lens) Mean± S.D	MDA (nmol/gmwt) Mean± S.D	SOD (Units/mg protein) Mean± S.D	Catalase (Units/mg protein) Mean ± S.D	GSH- Px Units/mg protein Mean± S.D	GSH-Rx Units/mg protein Mean± S.D
H_2O_2Gr.	740 ± 41.0	193 ± 60.01	19.70 ±4.14	0.35 ± 0.18	0.49 ± 0.10	21.8±12.6	16.43± 4.10
H_2O_2+ Proline (10mM)	690± 30.35 **P<0.001**	221.1± 42.67 **P<0.001**	8.97±2.81 **P<0.001**	0.87± 0.45 **P<0.001**	0.78± 0.46 **P<0.01**	27.58± 15.64 **P<0.01**	25.47± 15.59 **P<0.01**
H_2O_2+ **Tyrosine (100µM)**	685±28.40 **P<0.001 F=10.2**	216.6± 41.56 **P<0.05 F= 7.09**	10.41±3.55 **P<0.001 F=81.09**	0.84± 0.26 **P<0.001 F=18.12**	0.98± 0.60 **P<0.01 F= 2.94**	32.29± 18.44 **P<0.01 F=2.87**	27.20± 13.0 **P<0.001 F=2.81**

Table 2 shows statistically significant rise in Total soluble proteins after the addition of Proline (221.1± 42.67 mg/lens) and Tyrosine (216.6± 41.56 mg/lens)as compared with cataractous lens group and significant decrease observed in wet lens and MDA levels after the addition of Proline (690± 30.35mg, 8.97±2.81 nmol/gmwt) and Tyrosine(685±28.40mg,10.41±3.55 nmol/gmwt).

We also found that there was statistically significant increase in specific activity of Antioxidant enzymes after the addition of Proline (0.87± 0.45, 0.78± 0.46, 27.58± 15.64, 25.47± 15.59 in U/mg protein) and Tyrosine (0.84± 0.26, 0.98± 0.60, 32.29± 18.44, 27.20± 13.0 in Units /mg protein) as compared to the cataractous lens group.

Z' test was used to find the difference between means of both groups. ANNOVA was applied for compairing specific activity of antioxidant enzymes in both H_2O_2 and Endogenous antioxidant groups.

Discussion

Oxidative stress is one of the important factors involved in pathogenesis of cataract. The lens has antioxidant protection system i.e. Antioxidant nutrients & antioxidant enzymes to neutralize the free radicals [4].

In the present study, MDA levels are significantly raised in experimental cataractous lenses, which could be due to increased free radical generation & depletion of cellular defense mechanism [5, 6].

Decreased concentration of lens soluble proteins leads to insolublisation of soluble proteins which may be due to oxidative insult [7].

Present study shows decreased levels of antioxidant enzymes in cataractous lenses. But The addition of endogenous antioxidants (Proline&Tyrosine) shows marked increase in the enzyme levels. The toxic effect of reactive oxygen species may be neutralized by antioxidant defenses.

Proline protects lens proteins against aggregation and prevents GSH depletion caused due to oxidative stress [8]. Thus anticataract effect of proline may be related to its intrinsic ability to protect and restore the activities of lens enzymes.

Its ability of quenching toxic oxygen free radicals could be an additional therapeutically useful property in prevention of cataract.

Phenol group of Tyrosine performs vital antioxidant function inside lipid bilayer and protect
cells from oxidative destruction by scavenging superoxide anion radical and H_2O_2 radical [9].

Protein tyrosine phosphorylation is an important event in cell signal transduction process and phosphatidyl inositol 3-kinase (PI-3K) is also an intracellular signal mediator, plays key role in cellular function. So this phosphorylation process and activation of PI-3K are altered during cataract formation.

Conclusion

Endogenous antioxidants such as Proline and Tyrosine could be more effective in dealing cataract caused due to oxidative stress.

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