International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 1206-1212
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

HISTOPATHOLOGICAL AND GENE EXPRESSION STUDIES ON SODIUM SELENITE INDUCED CATARACT IN WISTAR ALBINO RATS USING *PERSEA AMERICANA* & *ACTINIDIA DELICIOSA* ETHANOL EXTRACTS

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ABSTRACT

Aim: *Persea americana and Actinidia deliciosa ethanol fruit extracts were used in determining the oxidative stress cataract induced by sodium selenite in Wistar albino rats.* ***Method:*** *The experimental rats were grouped into 6: Group I – Normal control, Group II – sodium selenite cataract induced animals, Group III – Persea americana extract co-treated to sodium selenite cataract induced animals, Group IV – Actinidia deliciosa extract co-treated to sodium selenite cataract induced animals, Group V – Persea americana and Actinidia deliciosa extract co-treated to sodium selenite cataract induced animals. Group VI – standard Ascorbic acid co-treated to sodium selenite cataract induced animals.* ***Results and conclusion****: Histopathology of the organs such as eye, lens, liver, spleen and heart were observed. Gene expression and protein expression of experimental animals were studied. Histological analysis of the Eye, lens, liver, spleen and heart in the Lens of Group I, Group III, Group IV, Group V and Group VI treated animals showed normal appearance. Group II administered with sodium selenite showed abnormal appearance of lens. An upregulated expression of α Crystallin, β Crystallin, m-Calpain and HSP-70 was observed in lens of rats with sodium selenite induced cataract. A significant down regulation of mRNA expression patterns of α Crystallin, β Crystallin, m-Calpain and HSP-70 was observed in control and in Persea americana and Actinidia deliciosa treated animals. When these fruits were taken in combination, they are more active in reducing the oxidative stress.*

KEY WORDS

Oxidative stress, Sodium selenite, ∞ Crystallin, β Crystallin and m-Calpain.

INTRODUCTION

Oxidative stress induced cataract is due to the oxidation of the critical sulfhydryl groups which initiates cataractogenesis [1]. Cataract causes several biochemical processes such as altered epithelial metabolism, calcium accumulation and proteolysis, insolubilization of protein, phase transition and opacification [2]. The alpha-crystallin is a soluble protein involved in refractive power in the lens and a chaperone-like function involved in binding of unfolded lens proteins. Alpha B-crystallin is also found outside the lens. Crystallins are over-expressed in response to stress, point mutation in alpha-crystallins of humans resulted deficit function, and cause cataracts [3]. Crystallin levels are elevated in several age-related degeneration diseases wherein oxidative stress is implicated as a major event in their pathogenesis [4].

Activation of CAP, and subsequent degradation of nuclear proteins, may be causes of selenite cataract [5]. Calpains are involved in cell mobility, cell cycle progression, potentiation in neurons , cell fusion in myoblasts, catalyzing the signal transduction pathway target proteins, regulate clotting , implicated in apoptotic cell death and appear to be an essential component of necrosis [6]. Histopathological examination revealed the signs of inflammation, haemorrhages, degeneration and rapid loss of normal cell architecture in the heart, liver, spleen and kidney of sodium selenite treated animals [7]. The present work is on histopathological studies of the organs, gene expression and protein expression of experimental animals.

MATERIALS AND METHODS

(i) Plant Collection and Authentication:

Edible fresh materials of *Persea americana and Actinidia deliciosa* were procured from the super market of Chennai, Tamil Nadu, India. The fruit samples were identified and authenticated as PARC/2013/2066 for *Persea americana* and PARC/2013/2067 for *Actinidia deliciosa* by Dr. P. Jayaraman, Botanist Prof., Plant Anatomy Research Centre, West Tambaram.

(ii) Extraction of Fruits

In the present study, we have carried out our work using ethanol extract of *Persea americana* and *Actinidia deliciosa* since our previous studies using ethanol extracts of fruits exhibits good radical scavenging activity than the aqueous extract and qualitative analysis of the extract has revealed the presence of phenols and flavonoids [8]. The fruits were rinsed using distilled water and shade dried. About 200 g of fruits were homogenized using Ethanol at an atmospheric pressure for 3 days by shaking at 100 rpm /min speed. The ethanol extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle in refrigerator until further use.

(iii) Animal study and Experimental Design

15 to 20 days old Wistar albino male rat pups weighing 30 ± 4 gm were purchased and maintained at Saveetha University, Chennai, Tamil Nadu, India with permission from Institutional Animal Ethics Committee (IAEC No. SU/BRULAC/RD/013/2014). The rat pups were induced for cataract with subcutaneous injection of sodium selenite, simultaneously then treated with both ethanolic fruit extracts of *Persea americana* and *Actinidia deliciosa*, standard ascorbic acid and maintained for 28 days. Animals were grouped into six of six animals each. **Group I (Control):** Received normal saline (0.3 ml /100g BW) at every 24 hrs for 28 days. **Group II (Cataract control):** Sodium selenite (30 µM / Kg BW) once induced and maintained for 28 days. **Group III:** Sodium selenite induced rats + *Persea americana* (1gm /Kg BW) **Group IV:** Sodium selenite induced rats + *Actinidia deliciosa* (1gm /Kg BW). **Group V:** Sodium selenite induced rats + *Persea americana* + *Actinidia deliciosa.* The Wistar albino rat pups of Group III, IV, V and VI received single dose of Sodium selenite (30 µM / Kg BW) before treatment. **Group VI (Standard):** Sodium selenite induced rats + Ascorbic acid (1gm /Kg BW). Groups were treated with fruit extracts at every 24 hrs for 28 days [**9, 10**]

(iv) Histopathological Studies

The organs were fixed in 10% formalin and were subjected to dehydration with ethanol of strength (70%, 80%, 90%, 95% and 100%) cleared in 2 changes of Xylene, impregnated with 2 changes of molten paraffin wax, and finally embedded in wax. Tissue sections of 4–5 µm in thickness were cut with a microtome and stained using Hematoxylin Eosin stain [**11**].

(v) Molecular Analysis

Gene expression was studied using the procedure [12]. Gene specific primers are retrieved from Primer Bank [13]. These primers are ordered from the MGH DNA Core facility [14]. All the primers are desalted and both UV absorbance and capillary electrophoresis are used to assess the quality of primer synthesis. The genes associated with cataract such as HSP 70, alpha and beta crystallin, Calpain–m and GAPDH were studied by Agarose Gel electrophoresis [15] and Western Blotting [16].

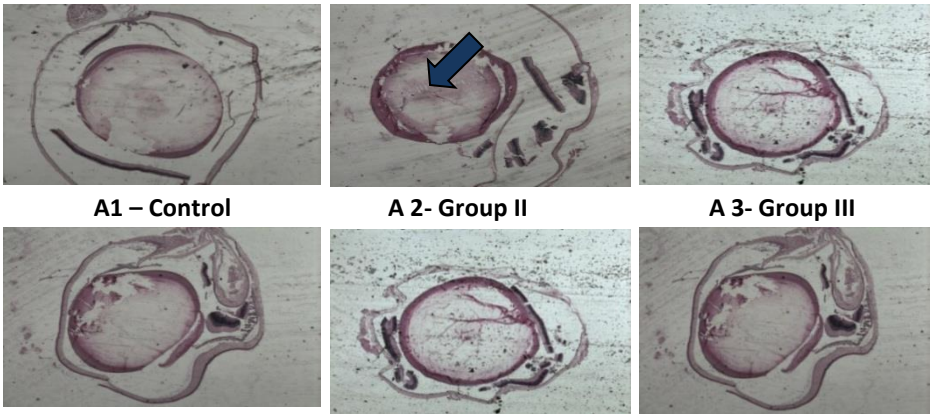
RESULTS

(i) Histopathology Analysis

Histopathology of Eye, Lens, Liver, Spleen and Heart of Group I (control) animals reveal normal architecture, whereas in Group II (sodium selenite induced cataract) abnormalities were observed. In Group III, Group IV, Group V and Group VI have no

changes for abnormality was observed in the organs (Plate A1 to A6; B1 to B6; C1 to C6; D1 to D6; E1 to E6).

Plate A1 to A6 Shows the Histopathology of Eyes of Experimental Animals



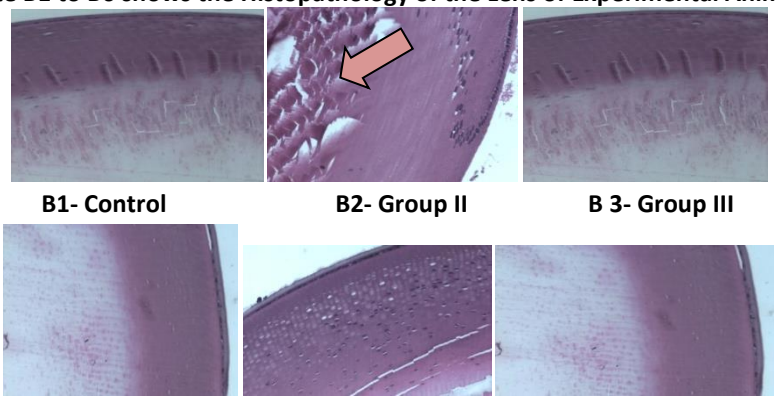
A1 – Control **A 2- Group II** **A 3- Group III**

A 4- Group IV **A5- Group V** **A6- Group VI**

Plate A1 shows normal histology of eye; Plate A2 shows accumulation of eosinophilic lens protein in eye; Plate A3, A4, A5 and A6 shows no changes of abnormality of eye.

The images are shown at 100x magnification

Plate B1 to B6 shows the Histopathology of the Lens of Experimental Animals



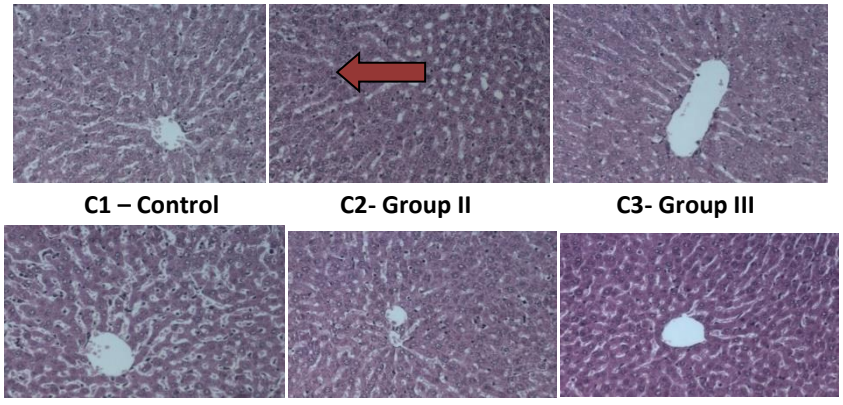
B1- Control **B2- Group II** **B 3- Group III**

B 4- Group IV **B5- Group V** **B6- Group V**

Plate B1 shows normal histology of lens; Plate B2 shows abnormal appearance of lens protein; Plate B3, B4, B5 and B6 shows no changes of abnormality of lens.

The images are shown at 100x magnification

Plate C1 to C6 shows the Histopathology of Liver of Experimental Animals



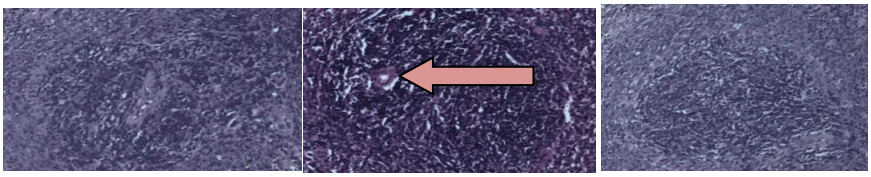
C1 – Control **C2- Group II** **C3- Group III**

C4- Group IV **C5- Group V** **C 6- Group VI**

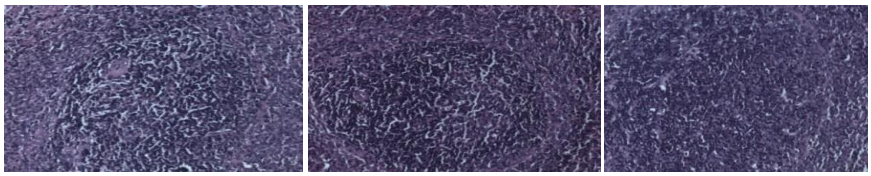
Plate C1 shows normal histology of liver; Plate C2 shows pericentral of perivnular mild chronic inflammation in liver; Plate C3, C4, C5 and C6 shows no changes for abnormality in liver.

The images are shown at 100x magnification

Plate D1 to D6 shows the Histopathology of Spleen of Experimental Animals



D1- Control **D2- Group II** **D 3- Group III**

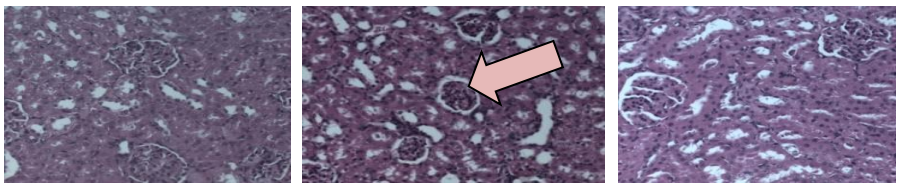


D4- Group IV **D 5- Group V** **D6- Group VI**

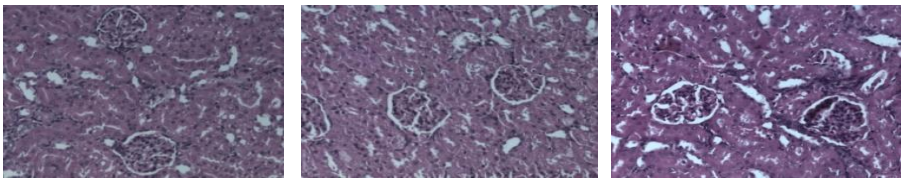
Plate D1 shows normal histology of spleen; Plate D2 shows white pulp expansion and scattered hemosiderin-laden macrophage in spleen; Plate D3, D4, D5 and D6 shows no changes for abnormality in spleen.

The images are shown at 100x magnification

Plate E1 to E6 shows the Histopathology of Kidney of Experimental Animals



E1 – Control **E2- Group II** **E3- Group III**

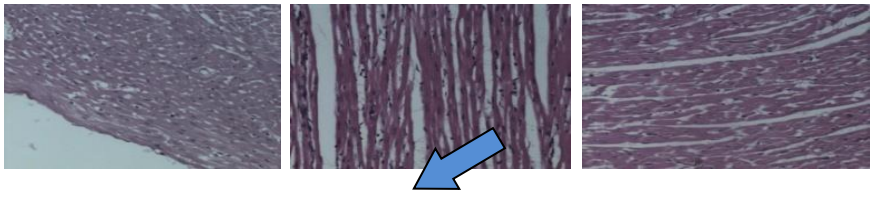


E4- Group IV **E 5- Group V** **E6- Group VI**

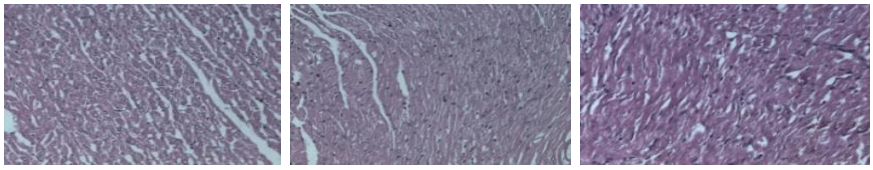
Plate E1 shows normal architecture of Kidney; Plate E2 shows inflammation in renal pelvis; Plate E3, E4, E5 and E6 shows no changes for abnormality in kidney.

The images are shown at 100x magnification

Plate F1 to F6 Shows the Histopathology of Heart of Experimental Animals



F1 – Control **F2 – Group II** **F 3 – Group II**

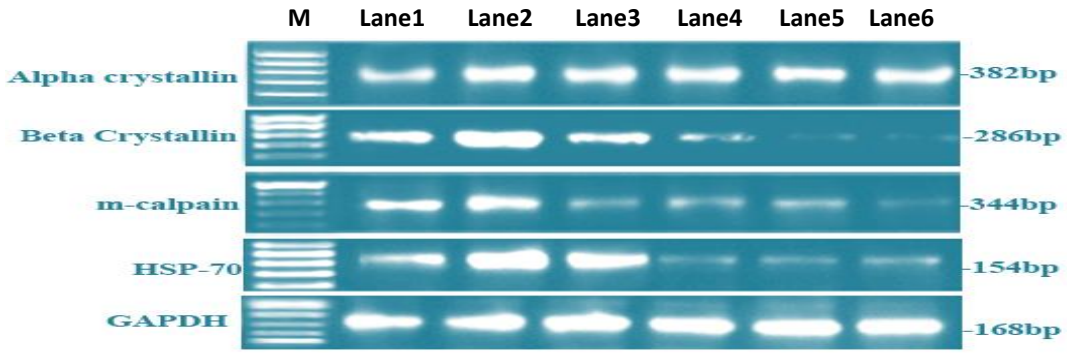


F4 – Group IV **F5 – Group V** **F6 – Group VI**

Plate F1 shows normal histology of Heart; Plate F2 shows abnormal appearance and inflammation in heart; Plate F3, F4, F5 and F6 shows no changes for abnormality in heart.

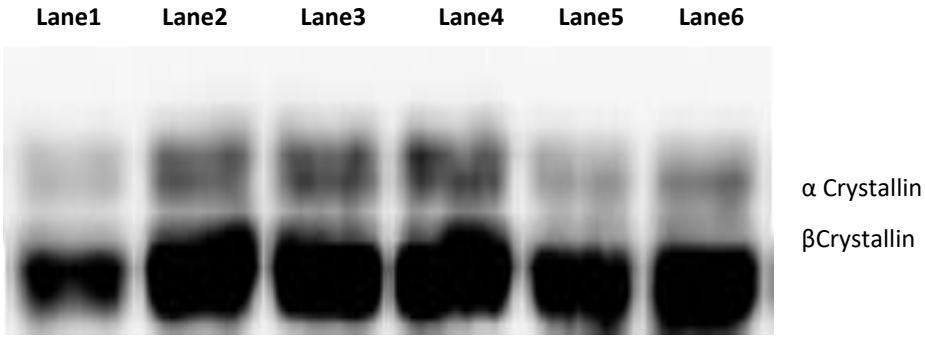
The images are shown at 100x magnification

Fig1. Shows the gene expression of Alpha crystallin, BetaCrystallin, m-calpain, HSP-70 and GAPDH.



M- Standard Marker; Lane 1 –Control group; Lane 2- Cataract induced group, Lane 3- *Persea americana* treated group; Lane 4-*Actinidia deliciosa* treated group, Lane 5- *Persea americana* and *Actinidia deliciosa* treated group & Lane 6- Ascorbic acid treated group.

Fig2. Protein expression of Alpha crystallin and BetaCrystallin



Lane 1 –Control group; Lane 2- Cataract induced group; Lane 3- *Persea americana* treated group; Lane 4- *Actinidia deliciosa* treated group; Lane 5- *Persea americana* and *Actinidia deliciosa* treated group & Lane 6- Ascorbic acid treated group

The selenite treated lenses shows abnormal lens morpholgy and disturbed epithelium. Later treated with Vitamin-E and selenite observed with normal lens morphology, anterio-posterior section of lens shows that the epithelium is undisturbed and observed as an intact layer [17]. Histopathological examination reveals degradation of normal cell architecture in the liver, kidney and eye lens of sodium selenite treated animals were restored with the simultaneous treatment with C-phycocyanin [18].

(ii) Gene Expression Study

The mRNA expression of α Crystallin, β Crystallin, m-Calpain, HSP-70 and GAPDH in lens of experimental animals detected by RT-PCR study. An upregulated expression of α Crystallin, β Crystallin, m-Calpain and HSP-70 was observed in lens Group II rats than in Group I- control lenses. GroupIII, Group IV and Group VI showed significant down regulation of mRNA expression patterns of α Crystallin, β Crystallin, m-Calpain and HSP-70 in lens compared to Group II animals. Whereas Group V has downregulated the mRNA expression of α Crystallin, β Crystallin, m-Calpain and HSP-70 in lens more significantly (Fig 1).

Enhanced calpain activity, regulated by CAPNS1, significantly contributes to platelet reactivity and thrombosis under hypoxic conditions [6]. Calpain 10 has been identified as a susceptibility gene for type II diabetes mellitus. The calpain inhibitor SJA6017 ameliorated *in vivo* selenite cataract formation in rats, thus stressing the significance of calcium homeostasis for maintaining healthy lens conditions [19]. Increased levels of αB-crystallin have been observed in many neurodegenerative disorders, tumors and diabetic conditions [20,21].

(iii) Protein Expression Study

The protein expression of α crystallin and β crystallin in lens of experimental animals was confirmed using Western Blotting Analysis. A higher level of expression of α - crystallin and β – crystallin proteins was observed in Group II lens of rats than in control Group I. Group III, Group IV showed significant reduction in protein expression of α crystallin and β crystallin in lens when compared to group II animals. Group V and Group VI further reduced the protein expression of α crystallin and β crystallin in lens (Fig 2).

The expression of αA and αB crystallin and heat shock protein in lenses showed higher levels in selenium treated than with curcumin treatment of selenium-induced cataractogenesis in Wistar rat pups [22]. It is a novel approach in modulating the chaperone activity of lens crystallins in selenite-induced cataract by a natural product [23]. Calpain-induced truncation of crystallins is a major cause of altered protein-protein interaction, which leads to protein precipitation [24].

CONCLUSION

The histopathological studies are the evidence of efficacy of fruit extracts as protectant. Gene expression of α crystallin, β crystallin, m Calpain and HSP-7 0 and protein expression in the lens of rats with sodium selenite induced cataract showed that both the fruit extracts has potent anticataract effect. There further needs to be screened by using GC-MS for their Bioactive constituents present in extract of these fruits.

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Received: 10.08.18, Accepted: 12.09.18, Published: 01.10.2018

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