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THERAPEUTIC EFFICACY OF *VERNONIA CINEREA* IN SELENITE INDUCED CATARACT MODELS

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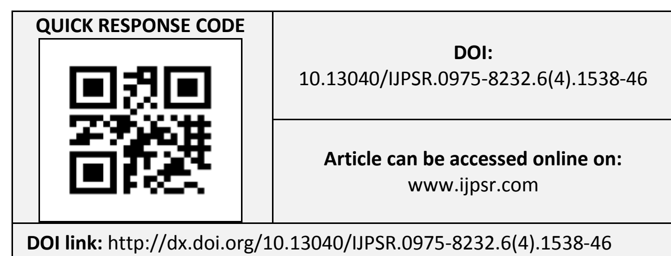
ABSTRACT: We have evaluated the efficacy of methanolic extract of *Vernonia cinerea* (MEVC) in selenite induced cataract using Sprague Dawley rats. Previous reports suggest that phytochemicals or natural plant products retard the process of cataractogenesis by scavenging free oxygen radicals. Hence, the present study sought to assess the potential of MEVC on *in vivo* selenite induced cataract models. The antioxidant activity was comparatively studied using DPPH radical scavenging and FRAP assay. MEVC exhibited higher DPPH radical scavenging activity as well as reducing power assay. In this study, cataract was induced by a single subcutaneous injection of sodium selenite (4µg/ g body weight) on rat pups. MEVC was administered orally from 8th day upto 21st day at a concentration 5 µg/g body weight. Cataract was visualized on 16th day with the help of an ophthalmoscope and later on with the naked eye. On the 30th day, rats were euthanized by sodium pentothal injection, lenses were excised and the biochemical parameters such as activity of catalase (CAT), Ca^{2+} ATPase and the content of reactive oxygen species (ROS), lipid peroxidation products (TBARS) were estimated and found effective in the treatment of cataract. We have also carried out experiments to assess the non toxic nature of MEVC in liver, kidney and serum and observed no significant variation in the GOT and GPT levels

INTRODUCTION: Visual disability is a common condition globally, affecting approximately 45 million and more than a third of blindness is caused by cataract. Approximately 25% of the population over 65 and about 50% over 80 has serious loss of vision because of cataract [1]. Cataract is a clouding of the lens of the eye that can impair vision. At present, the most effective treatment of cataract is surgical extirpation of opaque lens, but it is expensive and not free from risk factors [2]. Pharmacological intervention that prevents or slows progression of cataractogenesis has a significant health impact.



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Several studies reported that natural antioxidants and herbal drugs have potential anticataract activity [3, 4, 5]. Loss of transparency during cataract formation results from a variety of complex metabolic and physiologic mechanisms. In the cell, reactive oxygen species (ROS) may initiate a surge of toxic biochemical reactions such as peroxidation of membrane lipids and extensive damage to proteins causing intracellular protein aggregation and precipitation and eventually leading to lens opacification [6].

Oxidative stress leads to the production of ROS implies, intracellular production of those oxygen intermediates threatens the integrity of various biomolecules in lens disturb cellular homeostasis through protein modification and lipid peroxidation [7]. In addition to cataract oxidative stress is associated in wide selection of disorders including ischaemia, reperfusion injury, neurodegenerative

disease, diabetes, inflammatory diseases and aging. We know lens has a well designed system of defense against oxidation. It uses both enzymatic (catalase, Ca^{2+}ATPase) and non enzymatic (level of TBARS, ROS content) defense system to neutralize free radicals. Intake of food containing micronutrients that scavenge oxidants acts as antioxidant and anticataractostatic potential [8]. Selenite overdose cataract is a rapid and convenient model of study in experimental cataract. Major events of selenite cataract are decreased enzymatic action, loss of calcium homeostasis, generation of reactive oxygen species, lipid peroxidation, insolubilization of proteins, decreased concentration of water soluble proteins. Of the above, ionic homeostasis has been implicated in selenite cataract and most other types of cataract.

Naturally occurring compounds like flavonoids, alkaloids, tannins, anthocyanins, terpenoids etc. are known to exhibit antioxidant activity contain highly active pharmacological compounds. These compounds isolated from a part or whole of the medicinal herb act as pharmaceutical drugs have therapeutic potential against cataract. *Vernonia ciner*ea (Linn.) known as little ironweed, is a common annual weed (Asteracea) with a wide range of geographical distribution.

The whole plant has great medicinal value in diverse traditional usage in different nations and also gets recognition in the Ayurvedas. It is used in decoction or infusion to treat fever. It provides remedy for spams of the urinary bladder and strangury, and is often combined with quinine to treat malaria. Chloroform extract exhibited antidiuresis [9] property. Sesquiterpene lactones, which possess antimalarial activity, have been isolated from the plant [10]. Both polar and non-polar fraction of the plant extract showed analgesic, antipyretic and anti inflammatory effect. Paste of stem is used to heal wounds, while flowers are traditionally used to treat conjunctivitis, arthritis.

Polar extract of *V.cinerea* is found to have antidiarrhoeal activity [11]. Antibacterial [12] and anti larval activity against filarial vector [13] was reported. CCl_4 fraction of methanolic extract possesses significant antioxidant properties [14]. Besides these, ancient Ayurvedic literature also suggests the ophthalmic property of *V.cinerea*. Hence, the present study is aimed at systematically estimating the diverse therapeutic potentials of methanolic extract of *V.cinerea* (whole plant) with respect to antioxidant and anticataract properties.

MATERIAL AND METHODS:

Materials:

All the biochemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, USA) and other chemicals and solvents of analytical grade were from SRL Chemicals (Mumbai, India).

Plant material:

Whole plant of *Vernonia cinerea* was collected from Thiruvananthapuram District, Kerala State, India. The reference sample for the plant was kept in the herbarium of University of Kerala, Accession Number 5809.

Preparation of Plant extract:

200g of *V.cinerea* were taken in a round-bottomed flask, 80% methanol was added such that it covered the material and refluxed in a water bath at 65^0C for 24h. The supernatant was removed and the extraction was repeated twice. The extract was cleared of low polarity contaminants, such as fats, terpenes, chlorophyll by repeated extraction with petroleum ether. Methanolic extract contained the bulk of polyphenols and this extract was evaporated in vacuum. The non toxic extract was dissolved in water and employed for the *in vivo* studies.

Animal Experiments:

Sprague-Dawley rat pups at 8-10 days postpartum were housed along with their mother in polypropylene cages under a day/night cycle of 12 h, at 25±1°C room temperature. The rats received laboratory chow (Hindustan Lever Ltd., India) and distilled water.

All ethical guidelines were followed for the conduct of animal experiments in strict compliance with the Institutional Animal Ethical Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India [Sanction No: IAEC-KU-40/2011-'12-BC-AA(25)]. The rat pups

were grouped as follows with 12 pups in each group-

G I - Control (normal laboratory diet)

G II - Selenite-induced (4 µg/ g body weight) + normal laboratory diet

G III - Sodium selenite + MEVC (5 µg/ g body weight) + normal laboratory diet

G II, III were given a single subcutaneous injection of sodium selenite (4 µg/g body weight) on the 10th day, while rats of G I were injected with sterile water [15]. MEVC was administered as orally from 8th day upto 21st day at the concentration 5 µg/g body weight. Cataract could be visualized from the 16th day with the help of an ophthalmoscope and later on with the naked eye. On the 30th day, rats were euthanized by sodium pentothal injection, lenses were excised and the experiments conducted.

Toxicity examination:
Activity of SGOT/GOT was measured by the method of Reitman and Frankel [16]. 1 ml of substrate [dissolve 0.3 g L-Aspartate and 50 mg of oxoglutarate in 20-30 ml of phosphate buffer (0.15 M, pH 7.5) and to this add 10% NaOH (1.1 ml) to bring the pH to 7.5. Make up the volume to 100 ml with phosphate buffer] was taken in two tubes each and incubated at 37 °C for a few minutes. To one tube (Test), add 0.2 ml of serum/tissue homogenate and shaken gently. Exactly after 1h, add 0.07 ml of aniline-citrate reagent (Dissolve 50 g citric acid in 50 ml of distilled water and to this add an equal volume of redistilled aniline) to both tubes and 0.2 ml serum/tissue homogenate to the second tube (Blank). After 20 minutes, add 1 ml of DNPH (Dissolve 200 mg of 2-4-dinitrophenyl hydrazine in 85 ml of concentrated HCl and make up to one litre with distilled water) reagent to both tubes and incubate for another 20 minutes.

Remove the tubes from the bath and add 10 ml of 0.4 N NaOH and read absorbance at 520 nm after 10 minutes against reagent blank taking distilled water instead of serum/tissue homogenate. A pyruvate (1%) standard is prepared and activity calibrated using standard activity chart. In the measurement of SGPT/GPT, 2ml of substrate (Dissolve 5.0 g of DL-alanine and 20 mg of oxoglutaric acid in 20-30ml of phosphate buffer and add 0.5 ml of 10% NaOH to bring the pH to 7.5, can be stored in a refrigerator with a few drops of chloroform) was taken in two tubes each and incubated at 37 °C for a few minutes. The following procedure is taken in the case of the measurement of SGOT/GOT.

DPPH free radical scavenging activity:
The DPPH radical scavenging activity was measured by the method of Blois [17]. 0.1 mM solution of DPPH in ethanol was prepared and 0.1 ml of this solution was added to test solution at different concentrations (20 µM - 220µM) and made up to 3.0 ml with appropriate solution of ethanol-water to get 90% ethanol water system. After incubation for thirty minutes, the absorbance was measured at 517 nm. A system devoid of the MEVC served as control. All determinations were performed in triplicate. The IC_{50} value (concentration of sample corresponding to 50% inhibition of absorbance) was used to compare DPPH scavenging activity. Lower the IC_{50} value, higher the free radical scavenging activity.

Ferric reducing antioxidant power (FRAP) assay:
FRAP assay was performed according to the methods of Benzie and Strain [18] with slightly modification. An amount of 200 µl extracted samples were mixed with 3 mL FRAP reagent in test tubes and undergoes vortex. Blank samples were prepared for both methanol and deionized water extracted samples. Both samples and blank were incubated in water bath for 30 minutes at 37°C and the absorbance of the samples was determined against blank at 593 nm using aqueous solution of $FeSO_4.7H_2O$ as standard. The values obtained were expressed as µM of ferrous equivalent per gram of freeze dried sample.

Estimation of the activity of Catalase:
Activity of catalase was measured by the method of Aebi [19]. Reaction mixture containing 2.0 ml of enzyme preparation (in 50 mM phosphate buffer, pH 7.0) and 1.0 ml of 30 mM H2O2 (in 50 mM phosphate buffer, pH 7.0) was prepared. A system devoid of the substrate (H2O2) served as the control. Reaction was started by the addition of the substrate and decrease in absorbance monitored at

240 nm for 30 seconds at 25°C. The difference in absorbance per unit time was expressed as the activity. One unit is defined as the amount of enzyme required to decompose 1.0 µmole of hydrogen peroxide per minute at pH 7.0 and 25°C.

Estimation of the activity of Ca^{2+}ATPase:

The activity of Ca^{2+} ATPase in the lens samples was measured by the method of Rorive and Kleinzeller [20]. 0.25 ml of Tris – HCl buffer (0.4 M, pH 7.4) and 0.25 ml of 40 mM ATP (Tris salt) are taken in three test tubes kept in ice. To tubes 1 and 3, 0.25 ml of 40 mM $CaCl_2$ was added. At time zero, the reaction was started by the addition of 0.25 ml of enzyme extract to tubes 1 and 2. The volume in all the tubes was adjusted to 2 ml with distilled water. The tubes were incubated at 37°C with gentle shaking for 30 min. (Under these conditions, the release of phosphate will be linear for up to 60 min). The reaction was stopped by placing the tubes in ice and addition of 0.4 ml of ice-cold 35% TCA.

The tubes were then centrifuged for 10 min at 10,000 rpm in a refrigerated centrifuge. The supernatant can be kept frozen for later estimation of phosphate content. The phosphate content was estimated by the method of Fiske and Subbarow[21]. Because of the instability of ATP in acid solutions containing molybdate, tube 3 was used as a control to determine the phosphate liberated in the absence of enzyme. Results are expressed as micromoles of phosphate liberated/mg protein/h.

Determination of Thiobarbituric acid reactive substance (TBARS):

Levels of TBARS were estimated by the method of Niehaur and Samuelsson [22]. Tissue homogenate was prepared in 0.1 M Tris- HCl buffer. 1 ml of the homogenate was combined with 2 ml of TCA-TBA-HCl reagent and mixed thoroughly. The tubes were boiled for 15 min and the precipitate removed after cooling by centrifugation at 1000 x g for 10 min. Absorbance of the samples was read at 535 nm against a blank (without tissue homogenate). Results are expressed as µmoles/g tissue and calculated from the extinction coefficient of MDA.

Determination of reactive oxygen species (ROS)

The level of ROS was determined by the method of Davidson et al [23] which is expressed as fluorescence intensity (FI)/mg lens protein. A 10% (w/v) lens homogenate was prepared in phosphate buffer (100mM, pH 7.4) from this aliquots were taken for ROS estimation and protein estimation. In a tube 150- 200µl (5 mg protein) lens homogenate was taken, make up the volume to 990µl with homogenate buffer and 10.0µl of dichloro fluoroscien diacetate (1mM) was added to each tube including the buffer blank and allowed to incubate at 37°C for 30 min and centrifuged at 10,000 rpm for 15 min and the fluorescence of supernatant was recorded at an absorbance of 502 nm and an absorbance of 523 nm using a spectrofluorimeter.

Estimation of protein content:

The protein content of the samples was determined by the method of Lowry et al[24] using bovine serum albumin as the standard. To 0.04ml of sample, 1.0ml with distilled water and 5.0ml alkaline copper sulphate reagent were added and incubated for 10 min at room temperature. To this, 0.5ml of Folin's phenol reagent was added, incubated for 20 min at room temperature and absorbance measured at 670nm. Protein concentration was calculated from a standard curve taking 20-100µg BSA and measured against a reagent blank.

Statistical analysis:

All statistical calculations were carried out with statistical package for Social Sciences (SPSS) Software Program. The values were expressed as the mean ± SD. The data were statistically analyzed using one-way analysis of variance (ANOVA) and significant difference of means was determined using Duncan's multiple range tests at the level of $p < 0.05$[25].

RESULTS:

The DPPH radical scavenging activity of MEVC was used for the measurement of antioxidant property of MEVC as given in (**Fig. 1**). The IC_{50} (50% inhibition of radical) of MEVC was 32 µg/ml as compared to quercetin 27 µg/ml, indicating appreciable antioxidant potential of MEVC. FRAP assay for reducing power is widely used method to evaluate the antioxidant activity of polyphenols and may serve as a significant reflection of this efficacy. MEVC showed a concentration

dependent increase in the absorbance as an indication of its increased activity is shown in (**Fig. 2**).

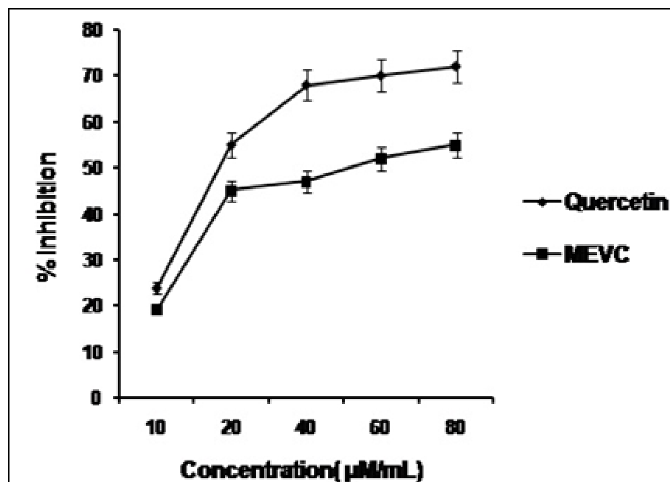

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY

Antioxidant activity of MEVC Antioxidants are able to reduce the stable DPPH radical to yellow-colored and the antioxidant power is indicated by the degree of discoloration which could be determined by measuring of a decrease in the absorbance at 517nm.

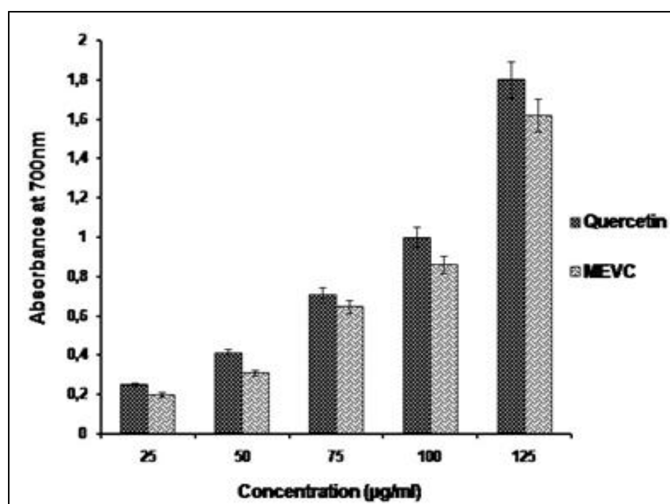

FIG. 2: FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Antioxidant activity of MEVC. The total antioxidant capacities of the plant extracts was determined by FRAP method. The principle of the assay is based on the reduction of ferric 2, 4, 6-tripyridyl-S-triazine [Fe (III)-TPTZ] to the ferrous 2, 4, 6-tripyridyl-S-triazine [Fe (II)-TPTZ] complex by a reductant at low P^H. This complex has an intense blue colour that can be monitored at 595 nm. Increases in absorbance indicate increase antioxidant property.

Table 1 Activity of GOT and GPT in Group I (G I), Group II (G II) and Group III (G III) Lenses of Rat.

TABLE 1: PARAMETERS EVALUATED TO ASSESS NON TOXIC NATURE OF MEVC IN RATS.

Parameters	G I	GII	GIII
SGPT	24.87± 2.46^a	24.04± 2.61^a	24.02± 2.16^a
SGOT	54.36± 4.17^b	54.83± 3.2^b	55.23± 3.29^b
Liver GPT	11.32± 1.38^c	10.16± 1.52^c	11.28± 1.26^c
Liver GOT	20.98± 1.04^d	20.76± 1.32^d	18.83± 0.94^d
Kidney GPT	5.38± 0.25^e	5.34± 0.28^e	5.58± 0.16^e
Kidney GOT	12.61± 0.83^f	12.74± 0.86^f	11.61± 1

Each value represents mean ± SD of six values. Activity of GOT and GPT expressed as IU/L. IU/L (GOT): µmoles of oxaloacetate liberated/min./mg protein. IU/L (GPT): µmoles of pyruvate formed/min./mg protein. G-I: Control, G-II: Selenite-induced (100µM Sodium selenite), G-III: Selenite-induced (100µM Sodium selenite) + MEVC (2.5 µg/ml). Results considered significantly different when $P < 0.05$. The alphabets a, b, c, d, e and f represents no significant difference indicate non toxic nature of MEVC.

Morphological examination of lens:

In our study, selenite administration (4µg/g body weight) in G II rat pups resulted cataractous lenses (100% cases). But in G I (control) no opacification of lenses. On the same way G III, which has received MEVC, resulted no opacification reveals its protective effect (**Fig. 4**).

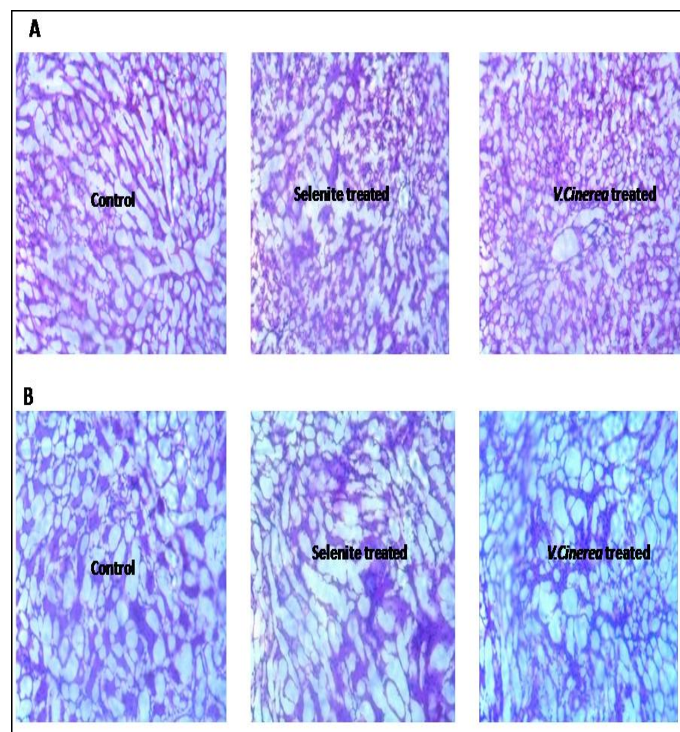

FIG. 3: TOXICITY STUDY.

Toxicity study was carried out to determine toxic effect of MEVC in liver, kidney and serum of experimental animals. Histopathological studies in liver (A) and kidney (B) showed no changes in the arrangement of cells. This reveals non toxic effect of MEVC.

FIG 4: MORPHOLOGICAL EXAMINATION OF LENS
The development of cataract in the rat lens was assessed by slit -lamp examination. All rat pups in Group II (which received s.c. injections of sodium selenite) exhibited dense opacification of the lenses. In contrast, rat pups in GIII (which received MEVC, along with sodium selenite) exhibited no opacification as that of control (Group I)

Cataract formation is initiated by the free radical hydrogen peroxide found in the aqueous humor. Catalase is a specific scavenger of hydrogen peroxide and its activity goes significantly reduced in cataract induced group (G II; $P<0.05$) when compared to the control, whereas the activity remains same as that of control in MEVC treated group. (**Fig.5A**). Increased lens calcium could be due to inhibition of outwardly directed Ca^{2+}ATPase pumps. Lens from rats injected with selenite showed a 50% decrease in Ca^{2+}ATPase activity. Inhibition of Ca^{2+}ATPase may be the most important mechanism for calcium accumulation in selenite nuclear cataract.

Thus, increased lens calcium may be due to oxidation of sulfhydryls and to other changes in the membranes caused by selenite, leading to inhibition of the Ca^{2+}ATPase pump and selective calcium permeability. The Ca^{2+}ATPase in selenite induced G II (4µg/g body weight) were significantly reduced when compared to that of G I ($P<0.05$) and G III (**Fig. 5B**).

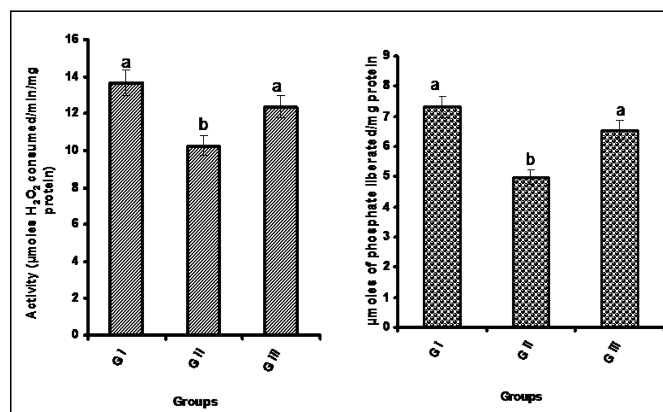

FIG 5: ACTIVITY OF CATALASE AND CA^{2+} ATPASE
G-I: Control, G-II: Selenite-induced (100µM Sodium selenite), G-III: Selenite-induced (100µM Sodium selenite) + MEVC (2.5 µg/ml). Activity of Catalase (A): µmoles H_2O_2 consumed/min/mg protein and Ca^{2+}ATPase (B): (µmoles phosphate liberated/mg protein/h). Comparison between groups, different alphabets indicate significant difference at $p<0.05$. Each value represents mean ± SD of six values.

Lipid peroxidation is a very complex process that involves the chain reaction of free radicals with polyunsaturated fatty acids. These reactions lead to rearrangements of double bonds in conjugated dienes, hydroperoxide generation etc. It is also associated with membrane damage and is a standard measure for free radical mediated cellular damage. The level of TBARS in selenite administered G II was increased when compared to that of G I. In contrast, the level of TBARS in G III, MEVC produced a reduction in its level compared to G II lenses ($P<0.05$) (**Fig. 6A**)

In lens, reactive oxygen species assault biomolecules including DNA, RNA, phospholipids and proteins leading to lipid peroxidation and depletion of the antioxidant enzymes. The extent of intracellular oxidation in lens homogenate was examined by measuring the level of ROS

production by DCFDA fluorescence intensity. Selenite induction caused an increase in lenticular ROS production in G II animals compared to G I. Whereas the pretreatment with MEVC before selenite induction maintained the activity in near normal levels compared to G II (**Fig. 6B**).

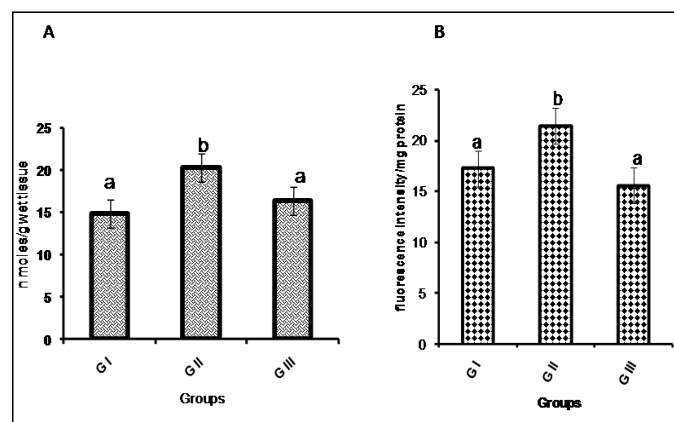

FIG 6: CONCENTRATION OF TBARS AND ROS CONTENT

G-I: Control, G-II: Selenite-induced (4 µg/g body weight), G-III: Selenite-induced + MEVC (5 µg/g body weight) treated. Concentration of TBARS (A): Values expressed as nmoles/100g wet tissue and ROS (B): values expressed as fluorescence intensity/mg protein. Comparison between groups, different alphabets indicate significant difference at $p<0.05$. Each value represents mean ± SD of six values.

DISCUSSION: Phytochemicals and natural products have been used as therapeutics in medicine because they are free from adverse side effects. Prevention of cataract has generated considerable interest in recent years. Although there is no way to cure or reverse the effects of cataract, prevention strategies can definitely slow the rates. Common cataract prevention methods include regular eye exams, eating foods rich in antioxidants etc.[26]. Selenite induced cataract has received much attention and was worked upon as a model system for oxidative-stress-induced cataract. Oxidative stress is involved as a common underlying mechanism and augmentation of the antioxidant defenses of the lens has been shown to prevent or delay experimental cataract. The selenite experimental model was selected because of its rapid, effective and reproducible cataract formation.

One of the objectives of cataract studies is hence to examine the feasibility of preventing its formation pharmacologically by use of metabolic antioxidants. A number of previous reports suggest therapeutic potential of *V. cinerea* against diverse types of ailments[27, 28, 29]. But the effectiveness of MEVC against selenite induced cataract in rat pups were presented in this report first. Phytochemicals and flavonoids present in plants can scavenge harmful reactive oxygen species and thus can form an antioxidant system.

In vitro radical scavenging activity by DPPH radical assay was conducted to ascertain the antioxidant potency of MEVC. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [30]. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm. This is an excellent method used to evaluate antioxidant property based on their ability to donate a hydrogen ion and is an excellent method used to evaluate antioxidants. The IC_{50} (50% inhibition of radical) of MEVC was 32 µg/ml as compared to quercetin 27 µg/ml, indicating appreciable antioxidant potential of MEVC.

FRAP assay is widely used method to evaluate the antioxidant activity of polyphenols. MEVC showed a concentration dependent increase in the absorbance as an indication of its increased activity is shown in **Fig 2.** Some phenolic compounds such as flavonoids and phenolic acids exhibited antioxidant activity through their reductive capacity in Fe^{3+}-Fe^{2+} system [31].

The antioxidant enzyme catalase plays a critical role in protecting cells from oxidative stress. Catalase is found virtually in all aerobic organisms. This enzyme serves in protect the cell from the toxic effect of small peroxides. In eye, catalase is concentrated in the epithelial layer. The main function of catalase may be to protect the lens from exogenous hydrogen peroxide produced by auto oxidation [32].

The inhibition of this enzyme may leads to the production of highly reactive hydroxyl radicals. Since it is a specific scavenger of hydrogen peroxide, its activity was significantly reduced in lenses of experimental animal group (Group II). The decreased catalase activity in cataractous

lenses may be due to the retro inhibition by H_2O_2 produced. Our study indicates that catalase was decreased in selenite induced cataract models, whereas the activity of this enzyme reached to normal level in MEVC treated group.

Selenite manifests its effect on lens by inducing oxidative stress in the critical sulfhydryl groups of proteins which could lead to the inactivation of membrane proteins like Ca^{2+} ATPase. In MEVC treated group, higher levels of Ca^{2+} ATPase activity were observed attributing to its protective effect. Thus phytochemicals or pharmaceutical drugs play an important role for the prevention of cataract. Previously published reports from our laboratory [33, 34] and other reports [35] agree with the obtained results. In the present study with animal model, a marked decrease in the activity of Ca^{2+} ATPase was found in the lens of selenite induced rats.

Lipid peroxidation due to oxidative stress is associated with membrane damage and is a standard measure for free radical mediated cellular damage. It was found that TBARS is the major breakdown product of lipid peroxidation which is significantly increased in human senile cataract [36]. Lipid peroxidation due to oxidative stress implicated in cataractogenesis because the accumulated peroxidation product induces fragmentation of soluble lens protein and damage vital membrane structure, correlating with an increase in lens opacity. The concentrations of the lenticular TBARS were elevated following selenite induction. The levels of TBARS reflect overall tissue lipid peroxidation. Lower level of TBARS in the MEVC treated group is an indication to the prevention of oxidative stress and lipid peroxidation, which may be due to antioxidant property of this extract.

Reactive oxygen species (ROS) are known mediators of intracellular signaling cascades. Excessive production of ROS may, however, lead to oxidative stress, loss of cell function, and ultimately apoptosis or necrosis[37]. A balance between oxidant and antioxidant intracellular systems is hence vital for cell function, regulation and adaptation to diverse growth conditions. Selenite induced group show high elevation of ROS when compared to that of normal. Decreased level of ROS was found in MEVC treated group. Therefore, the present investigation confirmed the protective action of *Vernonia cinerea* against oxidative stress in selenite induced cataract formation. Further studies were attempted to do for the isolation of active component present in *V.cinerea.*

CONCLUSION: The findings, therefore, suggest that *V.cinerea* has the therapeutic potential of lens against selenite induced cataract. It is possible that *V.cinerea* might be useful against lens damage caused by ROS generation under oxidative stress. It is also relatively nontoxic when given in small doses. Hence, these findings are considered pharmacologically significant; evaluation of active component from *V.cinerea* will certainly uncover novel therapeutic possibilities.

DISCLOSURE OF INTEREST: The authors declare that they have no conflict of interest concerning this article.

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