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Effect of the Fractions of *Coccinia grandis* on Naphthalene-induced Cataractogenesis in Rats

Muthuswamy Umamaheswari • Tapan Kumar Chatterjee*

Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India

Corresponding author: * **tkchatterjee81@yahoo.co.in**

ABSTRACT

Cataract, an opacity of the lens, is one of the major causes of blindness worldwide. The leaves of *Coccinia grandis* L. Voigt belonging to the family Cucurbitaceae are used in Indian traditional medicine for the treatment of gout, rheumatism, jaundice, eye infections, bronchitis, fever, skin eruptions, wounds, etc. The petroleum ether, chloroform, ethyl acetate, and residual fractions of the hydromethanolic extract of the leaves of *C. grandis* at a dose of 200 mg/kg b.w. were given orally to *Wistar* albino rats simultaneously with naphthalene (1 g/kg) for 28 days. Vitamin E (50 mg/kg) was used as the standard. Cataract progression due to naphthalene feeding was monitored using an ophthalmoscope and classified into 5 stages. At the end of the experiment, levels of malondialdehyde, lipid hydroperoxides, carbonyl and sulfhydryl content, enzymatic and non-enzymatic antioxidants in lens homogenate were measured. Administration of naphthalene produced a mature cataract and an increase in the opacity index. In addition, there was a significant increase in lipid peroxidation and protein carbonyl content and a decrease in protein sulfhydryl content and antioxidant enzymes when compared to healthy controls. Ophthalmoscopic observations indicated that simultaneous administration of the fractions delayed the onset and maturation of cataract. All the fractions (except the residual fraction) prevented the peroxidative damage caused by naphthalene, which is evidenced from the improved antioxidant potential. The effect produced by the chloroform fraction was almost comparable with the vitamin E-treated group. The leaves of *C. grandis* protected the lens against naphthalene damage which may be due to its antioxidant activity.

Keywords: antioxidant activity, cataract, lipid peroxidation, opacity index

INTRODUCTION

The normal lens is transparent and any congenital or acquired opacity in the lens capsule or substance, irrespective of the effect on vision, is a cataract (Kanski 2003). Cataract, a multi-factorial disease occurs mainly due to the formation of large protein aggregates in the lens. It is due to the post translational modifications of lens crystalline such as oxidation, glycation, Schiff's base formation, carbamylation, transamidation, phosphorylation and proteolysis leading to clouding of the lens (Unakar 1998). Cataract remains the leading cause of visual disability and blindness all over the world and the problem is more acute in developing countries. It is estimated that there are about 12 million blind people in India alone due to cataract (Gupta *et al.* 1997). It is associated mainly with the intensity of sunlight, especially of ultraviolet radiation. Even though surgical removal of cataractous lens with the use of corrective lenses has helped and provides a means to lessen the problem of vision loss, these procedures are expensive and not affordable for many in developing countries. Moreover, the lack of resources such as medical expertise, equipments, etc. has limited the availability of corrective measures for cataract related blindness in many countries (Varma 1991).

Naphthalene is a known cataractogenic agent and has been used for various experimental studies (Rees and Pirie 1967). Animals fed with naphthalene showed a characteristic subcapsular cataract with brown pigmentation, which serves as a potential model for human subcapsular senile cataract (Tao *et al.* 1991). Naphthalene is oxidized in the liver first to an epoxide and then is converted into naphthalene dihydrodiol. This stable component on reaching the eye gets converted enzymatically to dihydroxynaphthalene. Being unstable at physiological pH, 1-2 dihydroxynapthalene sponaneously auto-oxidises to 1-2 naphthoquinone and hydrogen peroxide (H_2O_2). It alkylates proteins, glutathione and amino acids and generates free radicals (Heyningen and Pirie 1967).

Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants. Many researchers have examined the effect of plants used traditionally by indigenous people to treat diseases of the eye. *Coccinia grandis* L. Voigt., belonging to the family Cucurbitaceae, is commonly known as "Ivy gourd" in English. It is a tropical plant found in many countries of Asia and Africa. The roots, stems, leaves, and whole plant of *C. grandis* are used in the treatment of eye infections, jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, allergy, syphilis, gonorrhoea, etc. (Kirthikar and Basu 1987; Wasantwisut and Viriyapanich 2003). The leaves of this species are widely used in Indian folk medicine for reducing the amount of sugar in urine of patients suffering from diabetes mellitus. Venkateswaran and Pari (2003) suggested the use of this plant in the treatment of diabetes. The crude hydromethanol extract of the leaves of *C. grandis* has been reported for its xanthine oxidase inhibitory and hypouricaemic activities (Umamaheswari *et al*. 2007). The various fractions of the hydromethanol extract of the leaves of *C. grandis* possessed significant *in vitro* antioxidant (Umamaheswari and Chatterjee 2008a) and *in vitro* xanthine oxidase inhibitory activities (Umamaheswari and Chatterjee 2008b). To our knowledge, there are no available reports on the effect of leaves of *C. grandis* for its anticataract activity. Hence, the objective of the present work was to evaluate the anticataract activity of the various fractions of *C. grandis* hydromethanol leaf extract against naphthalene-induced cataractogenesis in rats.

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Original Research Paper

MATERIALS AND METHODS

Experimental animals

Wistar albino rats of either sex weighing between 150-200 g were used for the study. The animals were housed in polypropylene cages inside a well-ventilated room. The room temperature was maintained at 23 ± 2°C with a 12 h light/dark cycle. The animals were fed with commercial rat feed pellets and provided with drinking water *ad libitum*. All animal procedures described were reviewed and approved by the University Animal Ethics Committee.

Drugs and chemicals

Naphthalene and vitamin E were obtained from SD Fine chemicals, Mumbai, India. Liquid paraffin was obtained from Fisher Chemicals Ltd., Chennai, India. Thiobarbituric acid, trichloro acetic acid, butylated hydroxytoluene, oxidized glutathione; epinephrine and DTNB were obtained from Sisco Research Laboratories Pvt., Ltd., Mumbai, India. 2-2'dipyridyl and *O*-dianisidine were obtained from Himedia Laboratories Ltd., Mumbai, India. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Plant material

Leaves of *C. grandis* were collected from approximately six-month-old plants in the vegetative season from Coimbatore district, Tamil Nadu, during May, 2006. The plant was identified and authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, India, where a voucher specimen (No. BSI/SC/5/23/06-07/Tech. 1951) of the plant has been deposited in the herbarium.

Preparation of extract and fractionation

Fresh leaves of the plant were dried in shade at room temperature and powdered mechanically and sieved through a No. 20 mesh sieve. About 500 g of the leaf powder was extracted with 2.5 L of methanol: water (7:3) by maceration at room temperature for 4 h using a mechanical shaker. The hydromethanol extract (27% w/v) was partitioned separately against petroleum ether, chloroform and ethyl acetate separately in the order of increasing polarity. The fractions were dried at 40°C under vacuum and the percentage yield of the fractions was petroleum ether (2%), chloroform (1.2%), ethyl acetate (1.4%) and residual fractions (18%).

Experimental protocol

An experimental model of cataractogenesis was induced in rats by feeding naphthalene at a dose of 1 g/kg orally for 28 days. Animals were divided into 7 groups consisting of six animals each. Group I received liquid paraffin (10 ml/kg b.w. orally) and served as the solvent control. Group II received naphthalene (1 g/kg b.w., orally) and served as the cataract control (Gupta 2004). Groups III-VI received petroleum ether (PEF), chloroform (CF), ethyl acetate (EAF), and residual (RF) fractions of *C. grandis*, respectively at a dose of 200 mg/kg b.w., orally simultaneously with naphthalene. Group VII received Vitamin E (50 mg/kg b.w., orally) with naphthalene and served as the positive control. All drugs were administered for a period of 28 days.

Examination of the eyes

Ophthalmoscopic examination of the eyes was performed after dilating the pupil with 1% tropicamide solution. The lenses were examined and graded twice a week during the first two weeks and thereafter at weekly intervals. Cataract formation was scored (Sippel 1996) according to the following stages: stage 1: clear normal lens; stage 2: peripheral vesicles; stage 3: peripheral vesicles and cortical opacities; stage 4: diffuse central opacities; stage 5: mature cataract.

Cataract formation was considered complete (stage 5) when the red fundus reflex was no longer visible through any part of the lens and the lens appeared dull white to the naked eye.

% Incidence of cataract was calculated using the formula (Fukushi *et al.* 1980):

$$\% \text{ Incidence} = \frac{\text{No. of animals in each stage}}{\text{Total No. of animals}} \times 100$$

Opacity index was calculated using the formula (Vats *et al*. 2004):

$$\text{Opacity index} = \frac{\text{No. of eyes in each stage} \times \text{stage of the eyes}}{\text{Total No. of eyes}}$$

Preparation of the lens

After 28 days of treatment, blood was collected by retro orbital puncture under mild ether anesthesia. The serum was used for the assay of lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LH) by the method of Nichans and Samuelson 1986.

Immediately after blood collection, animals were sacrificed by cervical dislocation and lenses were dissected out, rinsed with ice-cold saline and a portion of each lens was preserved in 10% formalin for histopathological studies. Each pair of lens was homogenized in a glass homogenizer with a Teflon pestle using ice cold phosphate buffer (20 mM, pH 7.4) and centrifuged and the supernatant was used for the analysis. The amount of total protein present in the lens tissue was estimated by the method of Lowry *et al.* (1951). Lipid peroxidation as evidenced by the formation of TBARS and LH were measured by the method of Nichans and Samuelson (1986). The content of free carbonyl in the total lens proteins was determined by the procedure of Levine *et al.* (1990) using the 2,4-dinitrophenylhydrazine (DNPH) reagent. The content of sulfhydryl groups in lens proteins was determined using Ellman's procedure as modified by Altomare *et al.* (1997). Superoxide dismutase (SOD) activity was determined by the inhibition of autocatalyzed adrenochrome formation in the presence of the homogenate at 480 nm (Kakkar *et al.* 1984). Catalase (CAT) activity was estimated by the catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 and recorded at 254 nm (Sinha 1972). Gutathione reductase (GSSH) activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm (Racker 1955). Glutathione peroxidase (GPx) activity was measured by the procedure of Paglia and Valentine (1967). Peroxidase (Px) activity was measured spectrophotometrically by following the change in absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of H_2O_2 and enzyme (Lobarzewski and Ginalska 1995). The estimation of reduced glutathione (GSH) was based on the reaction of reduced glutathione with dithionitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm (Ellmann 1959).

Statistical analysis

Statistical analysis was carried out using GraphPad software (GraphPad InStat) by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM from six rats in each group. P values < 0.05 were considered significant.

RESULTS

Staging of cataract and opacity index in various experimental groups are given in **Tables 1** and **2**, respectively. Ophthalmoscopic examination of the eyes showed that the lenses of the normal control were in stage 1 throughout the duration of the experimental period. On the other hand, animals treated with naphthalene showed a varying degree of cataractogenic changes as evidenced by about 66.6% of animals in stage 4 and 33.3% in stage 5 on the 28th day of treatment. None of the animals treated with the petroleum-ether, chloroform and ethyl acetate fractions of *C. grandis* showed mature stage 5 cataracts on the 28th day. Treatment with the ethyl acetate fraction showed 33.3% of animals in stage 4; whereas the residual fraction showed 66.6% in stage 4 and 16.6% in stage 5. Treatment with the standard drug, Vitamin E showed that 16.6% of animals were in stage

Table 1 Effect of the fractions of *Coccinia grandis* on cataract genesis in rats.

Group	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
% Incidence of cataract on 7th day					
C	100	0	0	0	0
NC	16.66	66.66	16.66	0	0
PEF	83.33	16.66	0	0	0
CF	83.33	16.66	0	0	0
EAF	66.66	33.33	0	0	0
RF	66.66	33.33	0	0	0
VE	100	0	0	0	0
% Incidence of cataract on 14th day					
C	100	0	0	0	0
NC	0	50	50	0	0
PEF	16.66	66.66	16.66	0	0
CF	66.66	33.33	0	0	0
EAF	16.66	66.66	16.66	0	0
RF	0	66.66	33.33	0	0
VE	83.33	16.66	0	0	0
% Incidence of cataract on 21st day					
C	100	0	0	0	0
NC	0	0	33.33	66.66	0
PEF	16.6	33.33	50	0	0
CF	33.33	33.33	33.33	0	0
EAF	0	66.66	33.33	0	0
RF	0	33.33	50	16.66	0
VE	66.66	33.33	0	0	0
% Incidence of cataract on 28th day					
C	100	0	0	0	0
NC	0	0	0	66.66	33.33
PEF	0	33.33	66.66	0	0
CF	16.66	50	33.33	0	0
EAF	0	33.33	33.33	33.33	0
RF	0		16.66	66.66	16.66
VE	33.3	50	16.66	0	0

C = Control, CF = Chloroform fraction, EAF = Ethyl acetate fraction, NC = Naphthalene control, PEF = Petroleum ether fraction, RF = Residual fraction, VE = Vitamin E.

Table 2 Effect of the fractions of *Coccinia grandis* on opacity index in control and experimental animals

Group	7th day	14th day	21st day	28th day
C	1	1	1	1
NC	2.16	2.99	3.66	4.16
PEF	1.32	1.99	2.32	2.99
CF	1	1.16	1.49	2.16
EAF	1.82	1.99	2.5	3.65
RF	2.16	2.66	3.16	3.99
VE	1	1.16	1.32	1.83

C = Control, CF = Chloroform fraction, EAF = Ethyl acetate fraction, NC = Naphthalene control, PEF = Petroleum ether fraction, RF = Residual fraction, VE = Vitamin E.

3 and there was no diffuse central opacities (stage 4) or mature cataract (stage 5). Treatment with naphthalene showed an increase in the opacity index from 2.16 on the 7th day, 3.66 on the 21st day followed by complete opacification (opacity index 4.16) on the 28th day. The groups treated with petroleum-ether, chloroform and ethyl acetate fractions of *C. grandis* showed a decrease in the opacity index (2.99, 2.16 and 3.65 respectively) on the 28th day when compared to naphthalene control. The incidence of cataract in the residual fraction-treated group was lower (3.83) in comparison to the naphthalene control. There was a marked reduction in the opacity index (1.83) of the Vitamin E-treated group when compared to the fractions.

At the end of the experiment (28 days), there was a significant ($P<0.01$) decrease in the level of total protein and an increase in the level of malondialdehyde (MDA) and lipid hydroperoxides in both the serum and lens of naphthalene-treated animals when compared to normal control. Treatment with the petroleum-ether, chloroform and ethyl acetate fractions of *C. grandis* and Vitamin E simultaneously with naphthalene for 28 days caused a significant ($P<0.01$) decrease in MDA and lipid hydroperoxide levels and an increase in total protein content. The effect produced by the residual fraction on the above parameters was found to be insignificant ($P>0.05$) compared to the naphthalene control (**Table 3**).

Treatment with naphthalene led to a significant ($P<0.01$) increase in the free carbonyl groups of lens proteins as measured by the absorbance of DNPH bound to total lens protein. At the end of the experiment, significantly higher values of free carbonyls were observed in the lenses of naphthalene-treated rats when compared to healthy controls. An increase in DNPH-reactive carbonyls was accompanied by a significant ($P<0.01$) decrease in sulfhydryl content titrable by DNPH. Treatment with the petroleum-ether, chloroform, and ethyl acetate fractions of *C. grandis* significantly ($P<0.01$) decreased the carbonyl content and increased the sufhydryl content similar to the Vitamin E-treated group. The effect produced by the residual fraction on carbonyl and sulfhydryl contents was insignificant ($P>0.05$) when compared to the naphthalene control (**Table 4**).

Treatment with naphthalene for 28 days produced a significant ($P<0.01$) decrease in the enzymatic antioxidants like CAT, SOD, Px, GPx, GSSH and the non-enzymatic antioxidant, GSH when compared to normal control. Treatment with the fractions of *C. grandis* (except the residual fraction) significantly ($P<0.01$) restored the levels of both enzymatic and non-enzymatic antioxidants which is almost similar to the control group. The activity produced by the standard drug was found to be the similar to the groups tested (**Table 5**).

DISCUSSION

Cataract is one of the leading causes of visual disability often leading to blindness. It is an age-related phenomenon and oxidative stress plays an important role. The situation can be remedied surgically by extirpation of the cataractous lens. The limitations of cataract surgery have stimulated experimental cataract research in laboratory animals using drugs of natural origin owing to their comparative safety. Plants like *Pterocarpus marsupium*, *Trigonella foeneum-graceum* (Vats *et al*. 2004), *Ocimum sanctum* (Sharma *et al*. 1998) and also compounds of plant origin like curcumin

Table 3 Effect of the fractions of *Coccinia grandis* on serum and lens MDA, LH and protein in control and experimental animals.

Group	MDA (nmoles/min/mg protein)		LH (nmoles/min/mg protein)		Protein (mmoles/min/mg wet tissue)	
	Serum	Lens	Serum	Lens	Serum	Lens
C	0.63 ± 0.04	0.59 ± 0.06	0.64 ± 0.02	0.55 ± 0.05	253.04 ± 47.62	1833.30 ± 6.92
NC	6.00 ± 0.50 a	1.90 ± 0.27 a	5.70 ± 0.29 a	2.00 ± 0.11 a	97.00 ± 17.02 a	784.90 ± 17.0 a
PEF	1.31 ± 0.16 b	0.82 ± 0.07 b	1.58 ± 0.22 b	0.90 ± 0.66 c	199.28 ± 31.84 c	1017.27 ± 4.49 b
CF	0.75 ± 0.06 b	0.78 ± 0.03 b	0.83 ± 0.05 b	0.66 ± 0.05 b	209.70 ± 13.10 b	1577.27 ± 15.0 b
EAF	2.78 ± 0.24 b	0.99 ± 0.05 b	3.15 ± 0.09 b	0.96 ± 0.03 c	194.78 ± 20.76 c	967.34 ± 19.1 b
RF	5.37 ± 0.21 d	1.74 ± 0.13 d	4.92 ± 0.51 d	1.54 ± 0.11 d	136.82 ± 5.11 d	812.11 ± 11.55 d
VE	0.70 ± 0.01 b	0.64 ± 0.06 b	0.71 ± 0.01 b	0.61 ± 0.05 c	217.39 ± 16.46 b	1512.40 ± 20.0 b

Values are mean ± SEM; n = 6 in each group.
a = P <0.01 when compared to control; b = P <0.01, c = P <0.05, d = P >0.05 when compared to naphthalene control (One way ANOVA followed by Dunnett's test)
C = Control, CF = Chloroform fraction, EAF = Ethyl acetate fraction, NC = Naphthalene control, PEF = Petroleum ether fraction, RF = Residual fraction, VE = Vitamin E.

Table 4 Effect of the fractions of *Coccinia grandis* on protein carbonyl and sulfhydryl contents in lenses of control and experimental animals

Group	Carbonyl content (nmoles/mg protein)	Sulfhydryl content (nmoles/mg protein)
C	0.68 ± 0.04	78.44 ± 2.68
NC	2.00 ± 0.33 a	45.30 ± 2.23 a
PEF	0.94 ± 0.04 b	63.34 ± 2.03 b
CF	0.87 ± 0.02 b	69.39 ± 4.19 b
EAF	1.06 ± 0.09 b	62.04 ± 3.09 b
RF	1.40 ± 0.10 c	51.57 ± 3.27 c
VE	0.77 ± 0.01 b	71.22 ± 1.79 b

Values are mean ± SEM; n = 6 in each group.
a = P <0.01 when compared to control; b = P <0.01, c = P >0.05 when compared to naphthalene control (One way ANOVA followed by Dunnett's test)
C = Control, CF = Chloroform fraction, EAF = Ethyl acetate fraction, NC = Naphthalene control, PEF = Petroleum ether fraction, RF = Residual fraction, VE = Vitamin E.

(Suryanarayana *et al*. 2003), quercetin, myricetin (Mohan *et al*. 1998) and proanthocyanidins (Osakabe 2004) have been shown to delay experimental cataract.

Naphthalene cataract was induced in rats by administering naphthalene (1 g/kg) in liquid paraffin orally for 28 days. As examined by a slit-lamp ophthalmoscope, at the end of the 4th week, approximately 33.3% of the animals developed a mature cataract (**Table 1**). Naphthalene-treated animals showed varying degrees of cataractogenic changes as evidenced by an increase in the opacity index with complete opacification at the end of the 4th week resulting in stage 5 mature cataracts. Treatment with the petroleum-ether, chloroform and ethyl acetate fractions of *C. grandis* did not completely prevent the development of cataract but significantly attenuated the severity of cataractogenesis. Animals treated with the petroleum-ether and ethyl acetate fractions of *C. grandis* showed a delay in the development of cataract but to a lesser extent than the chloroform fraction, whereas the reduction in the opacity index produced by the residual fraction was found to be insignificant. We previously reported the antioxidant activity of the leaves of *C. grandis* (Umamaheswari and Chatterjee 2008a) and it is possible that this might have contributed to its anticataract activity, since the process of naphthalene cataract is characterized by increased oxidation in the lens.

Lipid peroxidation, an event caused by an imbalance between free radical production and antioxidant defense, may play an important role in the development of cataract. The end products of lipid peroxidation are the toxic compounds, namely MDA and LH, whose involvement in cataractogenesis has been suggested, mainly due to its cross-linking ability (Micelli-Ferrari *et al*. 1996). Lens MDA may be the result of lipid peroxidation of the lens cell membrane or may represent a consequence of its migration from the readily peroxidizable retina or from the central compartment. In order to elucidate the protective mechanism of the leaves of *C. grandis*, the serum and lens of experimental animals were examined for the extent of lipid peroxidation. In our study, naphthalene-treated animals showed an increase in MDA and LH in both serum and lens (**Table 3**). Treatment with the petroleum-ether, chloroform and ethyl acetate fractions of *C. grandis* simultaneously with naphthalene caused a significant (P<0.01) decrease in both serum and lens MDA and LH and an increase in total protein content. This effect produced was almost similar to the Vitamin E-treated group.

Free carbonyls and sulfhydryls were used as markers of oxidative modifications of lens proteins. Decomposition of lipid peroxide initiates the chain reactions that produce an increase in protein carbonyl content and a decrease in protein sulfhydryl content in the lens (Boscia *et al*. 2000). Sulfhydryl oxidation through disulfide cross-linking and molecular aggregation leads to protein precipitation and lens opacification. At the end of our experiment, biochemical analyses of eye lens proteins showed a significant elevation of carbonyl groups and a diminution of sulfhydryl groups in naphthalene-induced cataractous lenses in comparision to normal controls. Treatment with the fractions of *C. grandis* (except residual fraction) significantly decreased the carbonyl content and increased the sulfhydryl content compared to naphthalene control (**Table 4**).

Intraocular generation of reactive oxygen species like superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen may constitute a significant risk factor in the overall pathogenesis of cataracts (Nijveld *et al*. 2001). Endogenous antioxidant enzymes constitute the most effective way of protecting the lens against damage due to free radicals. Lens contains a high concentration of reduced glutathione (GSH), which can remove the free radical species such as superoxide anion, alkoxy radicals and hydrogen peroxide. The enzyme glutathione reductase (GSSH) catalyses the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH), thereby maintaining a constant level of GSH in a normal lens (Rathbun and Bovis 1986). Glutathione peroxidase (GPx) removes hydrogen peroxide (H_2O_2) while catalyzing oxidation of GSH to GSSG, thus providing the major line of defense against endogenous peroxides. A decrease in the level of antioxidant enzymes has been reported in cataractous lenses (Vani and Rawal 1996). Our results also indicated a significant decrease in the GSH level and the activities of GPx and GSSH in naphthalene treated animals as compared with the control group. However, in the groups supplemented with the fractions of *C. grandis*, there was a significant increase in GSH level which was substantiated with a parallel increase in the activity of GPx and GSSH, suggesting the protective mechanism of the fractions in response to free radicals (**Table 5**). The activity produced by the chloroform fraction was found to be the highest among the fractions tested and is almost similar to the Vitamin E-treated group.

In addition, the lens and the other eye tissues do contain SOD as well as CAT and Px. SOD is an enzymatic antioxidant that scavenges the superoxide anion and converts into H_2O_2, hence diminishing the toxic effect caused by this radical. The decomposition of H_2O_2 to H_2O is catalysed by CAT in association with Px. Thus, a decrease in the activities of both CAT and Px leads to an accumulation of H_2O_2. Treatment of rats with the fractions of *C. grandis* increased the activities of enzymatic antioxidants and prevented the accumulation of excessive free radicals from naphthalene

Table 5 Effect of the fractions of *Coccinia grandis* on tissue enzymatic and non-enzymatic antioxidants in control and experimental animals.

Group	Catalase (µmoles/min/mg protein)	GPx (nmoles/min/mg protein)	SOD (nmoles/min/mg protein)	GSSH (nmoles /min/mg protein)	Peroxidase (nmoles/min/mg protein)	GSH (nmoles/min/mg protein)
C	28.04 ± 1.64	183.23 ± 0.10	28.70 ± 0.76	32.57 ± 1.07	27.52 ± 1.00	70.50 ± 5.17
NC	10.60 ± 0.80 a	96.10 ± 3.82 a	9.90 ± 0.61 a	15.10 ± 1.41 a	11.00 ± 1.01 a	25.10 ± 1.30 a
PEF	19.91 ± 0.31 b	173.23 ± 2.83 b	19.57 ± 0.93 b	25.36 ± 1.29 b	17.63 ± 0.50 b	38.42 ± 1.38 b
CF	23.76 ± 1.39 b	177.65 ± 29.34 b	22.62 ± 1.54 b	27.74 ± 1.53 b	21.87 ± 0.98 b	68.76 ± 2.87 b
EAF	16.50 ± 0.89 b	162.90 ± 1.820 c	16.40 ± 0.73 b	21.49 ± 1.27 b	16.08 ± 1.00 b	32.61 ± 1.90 c
RF	11.12 ± 0.40 d	148.01 ± 5.710 d	10.11 ± 0.71 d	16.03 ± 0.66 d	13.18 ± 0.28 d	30.02 ± 1.51 d
VE	26.40 ± 2.27 b	178.20 ± 21.50 b	26.70 ± 2.26 b	31.14 ± 0.90 b	22.20 ± 0.83 b	69.45 ± 2.20 b

Values are mean ± SEM; n = 6 in each group.
a = P <0.01 when compared to control; b = P <0.01, c = P >0.05 when compared to naphthalene control (One way ANOVA followed by Dunnett's test)
C = Control, CF = Chloroform fraction, EAF = Ethyl acetate fraction, NC = Naphthalene control, PEF = Petroleum ether fraction, RF = Residual fraction, VE = Vitamin E.

intoxication (**Table 5**).

A number of scientific reports have indicated that antioxidants may be useful for prophylaxis or therapy against cataracts (Varma 1991). We have previously reported the *in vitro* antioxidant activity of the fractions of the leaves of *C. grandis* and the presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralising free radicals (Umamaheswari and Chatterjee 2008a).

To conclude, this study suggests that the leaves of *C. grandis* possess anticataract activity which might be helpful in preventing or slowing the progress of cataract formation. Further investigations on the isolation and identification of active components in the various parts of the plant may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

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