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Chemopreventive and remediation effect of *Hydrocotyl bonariensis Comm. Ex Lam* (Apiaceae) leave extract in galactose-induced cataract

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ABSTRACT

Ethnopharmacological relevance: Hydrocotyl bonariensis Comm. Ex Lam (Apiaceae) is being widely used in Western Nigeria in treating various symptoms of ophthalmic diseases; however scientific data in support of this medicinal use have not been reported.
Aim of the study: This study, investigated the efficacy of *Hydrocotyl bonariensis* leave extract in offering protection against experimental cataract and also examined its remediation effect when administered after cataract onset.
Materials and methods: Weanling albino rats fed with 30% galactose diet were used in the study. Mechanisms of action of the extract were investigated by measuring the degree of lens peroxidation, lens antioxidant status and lens protein concentration. Severity of cataract was determined by measuring the cataract index.
Results: The extract at 500 mg kg^{-1} reduced cataract index significantly and also reduced cataract progression when administered after cataract onset. Administration of this dosage also significantly reduced the degree of lens peroxidation, increased the level of reduced glutathione (GSH) and the lens catalase and superoxide dismutase activity. The extract also prevents protein insolubilization. Administration of the extract at 1000 mg kg^{-1} reduced cataract index and lens peroxidation but did not increase the antioxidant status significantly. Administration of the extract after cataract onset reduced cataract index, moderately increased percentage soluble protein above the value prior to the arrest of hypergalactosemia but did not increase the antioxidant status.
Conclusion: Our study suggests that *Hydrocotyl bonariensis* protects against galactose-induced cataract, and that administration of the extract after cataract onset reduced cataract progression but did not reverse cataractogenesis.

1. Introduction

Cataract, opacity of the lens, is the leading cause of blindness worldwide and is responsible for 50% of the blindness in underdeveloped countries. Nearly 50 million people in the world are blind due to cataract. The age-adjusted prevalence of cataract in West Africa is three times higher than in United States (Leske and Chylac, 1997). Data from The Ophthalmological Society of Nigeria (2005) indicates that about 1.2 million people in Nigeria are blind due to cataract and about 4.08 million people have low vision. The report also estimated that the number of blind and low-vision people would almost double by the year 2020 unless concerted action is taken.

The etiology of cataract is not fully understood, though oxidative damage to the constituents of the eye lens is considered to be a major mechanism in the development (Spector, 1991). Current evidence supports the view that cataractogenesis is a multifactorious process, in which combination of more closely linked events induce subtle, translational modifications in the lens structural proteins, enhancing their aggregation, fragmentation and precipitation, resulting eventually in lens opacification (Kyselova et al., 2005; Suryanarayana et al., 2004; Reddy et al., 2002; Hockwin et al., 2002; Stitt, 2001). Factors that have been implicated in increasing the risk of cataract include: aging, nutritional deficiencies, diabetes, sunlight, environmental factors and lack of antioxidants' consumption (Suryanarayana et al., 2004).

The human lens has a protein concentration of 33% of its wet weight. The lens proteins are of two types: the water soluble protein known as the crystallins which accounts for 90% of lens proteins and the water insoluble fraction (Thomas et al., 2004). Although there is a battery of other posttranslational modifications, accumulation

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of large amounts of insoluble protein derived from the otherwise soluble protein due to aggregation has been identified as the major biochemical mechanism in cataractogenesis (Reddy et al., 2002). The constituents of lens other than water and protein are quantitatively minor though they may be very important metabolically.

The goal of lens metabolism is the maintenance of transparency. In the lens, energy production largely depends on glucose metabolism. Three pathways: anaerobic metabolism, hexose monophosphates shunt and polyol (sorbitol–aldose reductase) pathways are utilized by the lens for glucose metabolism. The polyol pathway has been implicated as the primary cause of cataractogenesis in diabetics. The formation of hydroxyl radical was detected in sugar cataracts induced by galactose in rats using ESR spin-trapping method with a spin-trapping agent DMPO (Kubo et al., 1999). Polyol accumulation of lenses in the same group simultaneously peaked. It was suggested that hydroxyl radical was produced in proportion to polyol accumulation in the early cataract stage. Aldose reductase is the key enzyme for the polyol pathway. This enzyme has been found to play a pivotal role in the development of "sugar" cataracts (Pallavi, 2003). Aldose reductase reduces the unused glucose to sorbitol which is then oxidize to fructose. In uncontrolled diabetics, polyol pathway is highly favored which ultimately leads to production of sorbitol. An increase in the concentration of glucose contributes to an enhanced activity of the two enzymes used in the polyol pathway: aldose reductase and sorbitol dehydrogenase. With the increased activity of these two enzymes, there is a consequent reduction in the NADPH:$NADP^+$ ratio and the NADH:NAD^+ ratio (Giugliano et al., 1996). The decreased concentration of these cofactors may cause an inhibition in enzymes which are NADPH-dependent and lead to the shortage of NADPH available for many pathways it is involved in. Consequently, there may be decreased synthesis of reduced glutathione (GSH), nitric oxide, mynositol and taurine. These may cause an increase in free radical production which may lead to ischaemia (Brownlee, 2001). Galactose is also a substrate for aldose reductase, producing an alcohol galactitol, which accumulates in the lens. The result is the swelling of fibers, disruption of the normal cytoskeletal architecture and lens opacification (Thomas et al., 2004). Galactitol is not however a substrate for sugar alcohol dehydrogenase and thus accumulates rapidly, producing the same osmotic effects and the same consequence as sorbitol. Galactokinase is the first enzyme in the metabolism of galactose; the conversion of galactose to galactose-1-phosphate. Galactokinase deficiency results in galactosemia, galactosuria and cataracts. Cataracts form in galactokinase-deficient individuals because ingested galactose cannot be metabolized and is therefore available for reduction to galactitol (Ruihua et al., 2007).

No drug is available that has been proven to prevent or reverse the progression of senile cataract. New medical advances such as surgery and lens replacement have a high degree of efficacy, but are not available to or too expensive for most of the world's population. Recently, synthetic aldose reductase inhibitors have been studied for the treatment of diabetic cataract. However, these drugs are expensive and sorbinil; the most studied drug in particular, has considerable side effect. The use of traditional medicines, mainly derived from plant sources, has played a major part in the management of many chronic ailments (Swanston-Flatt et al., 1999; Grover et al., 2002). Moreover, there is a renewed interest in recent times to identify as many plant resources as possible because of their therapeutic values (Chang, 2000; Grover et al., 2002). A number of observational studies suggest that intake of foods containing micronutrients having antioxidant potential may be protective against cataract. In a study by Ansari and Srivastava (1990), antioxidant supplementation was found to inhibit the development of cataract in experimentally induced diabetic rats. Curcumin, a widely used spice in India has also been shown to have significant antioxidant activity both *in vitro* and *in vivo* and shows some anticataractogenic properties (AnilKumar et al., 2005).

Hydrocotyle bonariensis (large leave pennyworth), once a member of the family Apiaceae, now in the family Araliaceae and of the genus *Hydrocotyle* is an herbaceous, hairless, creepy, perennial plant, prostate, with creeping lateral stems (Knight and Thomas, 2004). They are mostly found in Africa and America. Its common name in West Africa, Nigeria is Karo. Its habitats are beach dunes, moist, open sandy areas, wet ditches and edges of ponds (Evans and Whitney, 1992; Linhart and Grant, 1996). Its medicinal use as emetics, diuretics and laxatives has been reported (Evans, 1992). The use of the leave of the plant in treating various symptoms of ophthalmic diseases is rapidly gaining popularity among some local people in Western Nigeria (Edeoga et al., 2005) and this is without scientific proof, since no data has been sighted in the literature to support this use. In a previous study, we have identified alkaloids, flavonoids, tannins, phenolic compounds and saponins as bioactive components of the leave of the plant (Ajani et al., 2008). Tannins and saponin, have been shown to have medicinal activity and exhibit physiological properties (Sofowora, 1993). A study by Goodarzi et al. (2006) reported that oral consumption of two flavonoids, quercetin and naringin could inhibit aldose reductase activity in lenses of diabetic rats. The inhibitory action of flavonoid against aldose reductase has been attributed to the capacity of flavonoid to chelate metals (Mira et al., 2002). In the present study we attempted to investigate the efficacy of the leave extract of *Hydrocotyl bonariensis* in preventing and/or reversing the development of cataract induced by dietary galactose.

1.1. List of abbreviations

The following are the abbreviations used throughout the text:

DMPO: 5,5-dimethyl-1-pyrolline-N-oxide
MDA: malondialdehyde
TBARS: thiobarbituric acid reactive substances
BSA: bovine serum albumin
ESR: electron spin resonance
NADPH: nicotinamide adenine dinucleotide phosphate (reduced)
NADH: nicotinamide adenine dinucleotide (reduced).
dUTP: deoxyuridine triphosphate

2. Materials and methods

2.1. Plant authentication and extraction

Mature fresh leaves of *Hydrocotyl bonariensis* were collected from a local garden and were authenticated at the herbarium of the Botany Department, University of Lagos, Nigeria. The herbarium voucher number is 13478. The leaves were then oven dried at 40 °C for 24 h. They were blended using a local kitchen blender, and 240 g of the blended leaves were soaked in 2500 ml of water at room temperature for 48 h. The extract was then sieved into a clean container and further concentrated using a rotary evaporator at 40 °C. The concentrated product was then lyophilized. The yield of the extract was 5.8%.

2.2. Experimental design and dietary regimen

Forty (40) male wistar albino rats (21 days old) having an average body weight of 30 g, bred in the animal house, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria, were used in the study. The rats were randomized into groups and treated as follows; Group A: rats in this group were sacrificed before the commencement of treatment (baseline); Group B: rats were fed with a normal stock diet based on the AIN-93

(Reeves, 1997) formula (10 rats); Group C: rats were fed with 30% galactose in the above diet (15 rats); Group D: rats in this group received the Group B diet and were administered with 500 mg kg^{-1} extract (5 rats); Group E: rats in this group received the Group B diet and was administered with 1000 mg kg^{-1} extract (5 rats). Animals were housed in individual cage in a temperature and humidity controlled room, having a 12-h light and dark cycle. All the animals had free access to their respective feed and clean drinking water. The treatment was carried out for four (4) weeks after which food was withdrawn from the animals overnight. They were then sacrificed by cervical dislocation after diethyl ether anesthesia. Their eyes were removed and lenses were dissected using posterior approach.

The remaining rats in Groups B and C were reassigned into groups labeled as follow: B1 (5 rats); C1 (5 rats), C2 (5 rats). All the rats were maintained on the AIN-93 stock diet for two (2) weeks. Groups B1 and C1 rats were placed on 0.1 ml normal saline while Group C2 rats were treated with 500 mg kg^{-1} dose of the extract. All administration was carried out orally as a single dose with the aid of an oral intubator.

2.3. Blood and lens collection

After two weeks treatment period, food was withdrawn from the rats overnight. They were then sacrificed by cervical dislocation. Their eyes were removed and lenses were dissected using posterior approach. They were then cleaned of blood and dirt, weighed and stored at −70 °C.

2.4. Animal care

The care of the animals was in accordance with the U.S. Public Health Service Guidelines (NRC, 1999) and approved by the Olabisi Onabanjo University, College of Health Sciences Animal Ethics Committee.

2.5. Preparation of crude homogenate

Lenses from 2 rats in each group were pooled together and homogenized in 10%, 100 mM ice-cold potassium phosphate buffer, pH 6.2. The homogenate was centrifuged at 15,000 × *g* for 30 min at 4 °C (Suryanarayana et al., 2004). All the biochemical parameters were analyzed with the soluble fraction of the lens homogenate except the lens MDA that was estimated in the total homogenate.

2.6. Cataract index and histological study

One eye from each rat was fixed overnight in 10% neutral buffered formalin. Tissue was routinely processed for paraffin embedding. Tissue was stained with hematoxylin and eosin (HE) and the selective tissue used for TUNEL staining (Yoshihiro et al., 2001).

To carry out the TUNEL staining, the formalin fixed tissue were extended on glass slides and treated with 10-fold diluted proteinase K (DAKO, Glostrup, Denmark). Apop DETEK (DAKO) was used for deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling reaction, and horseradish peroxidase-DAB in situ detection System (DAKO) was used for reaction of biotin-labeled dUTP with peroxidase–avidin complex. Bilateral eye specimen was evaluated in HE-stained tissue based on the presence of the following 7 items: (1) lens epithelial apoptosis, (2) lens epithelial desquamation, (3) multilayered spindle epithelium, (4) lens fiber swelling, (5) liquefaction/vascular change, (6) calcification and (7) bizarre nuclei of lens fiber cell. Each item was arbitrarily scored as: absent = 0; slight = 1; moderate = 2; severe = 3; very severe = 4. Cataract index is the sum of each score (Katsuji et al., 2002).

2.7. Biochemical analysis

Lens MDA was estimated as TBA reacting substances (TBARS) as described by Bhuyan et al. (1981). Reduced glutathione (GSH) was assayed for by the method of Beutler et al. (1963); catalase activity was assayed for by the method of Sinha (1972). The method of Del-Maestro et al. (1983) was used to determine superoxide dismutase activity. Protein content was determined by the method of Lowry et al. (1951) using BSA as standard.

2.8. Validation of biochemical analysis

For validation of the aforementioned assay, all the parameters were repeatedly analyzed in the lenses of healthy rats. Reproducible assay characterized by SEM and obtained in repeated determinations ($n = 5$) were noted and recorded as baseline value (A).

2.9. Statistics

All data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used to analyze the data. Comparisons between the groups were made at a two-sided alpha level of 0.05. $p < 0.05$ was considered statistically significant.

3. Result

3.1. Cataract index and histological study

Table 1 shows the severity of lens lenticular damage indicated by the cataract index. The result indicates that dietary galactose treatment significantly increase lens epithelial apoptosis, lens epithelial desquamation, and liquefaction/vacuolar change when compared with the normal control and the baseline value. Administration of the extract at the two tested doses simultaneously with the dietary galactose, reduced this increase. Withdrawal of dietary galactose only (C1) reduced the liquefaction/vacuolar change while administration of the extract simultaneously with the withdrawal (C2) significantly reduced all the parameters compared with the value prior to the withdrawal (C). When compared, the lenticular damages recorded when the extract was administered simultaneously with the dietary galactose withdrawal was reduced as copmpared to the damages recorded when the rats were treated with normal saline. After four weeks treatment period, a significantly higher cataract index was recorded in rats placed on high dietary galactose when compared with the normal control group (B) and the baseline value (A). The observed cataract index (following withdrawal of dietary galactose) as measured at the end of the sixth week treatment period was significantly lowered compared with the value observed at the end of the fourth week. Administration of the extract at the two tested doses simultaneously with the dietary galactose treatment significantly reduced the cataract index when compared with the group that was not treated with the extract. The observed cataract index in the group treated with 1000 mg kg^{-1} dose of the extract (E) was not significantly different from that observed in the group treated with 500 mg kg^{-1} (D). When dietary galactose was withdrawn from the rats, and the extract was simultaneously administered, the observed cataract index was significantly lowered compared with the value observed when the rats were fed with dietary galactose. However, the cataract index observed in the group placed on normal saline following the withdrawal of dietary galactose was significantly higher than that of the group placed on the extract. No observable index of cataract was recorded in the normal control group (B) at the end of the fourth week of study and after the sixth week of study. Similarly, none was observed before the commencement of the

Table 1
Result of severity of histological damages and cataract index of albino rat after treatment.

Group/treatment	Lens epithelial apoptosis	Lens epithelial desquamation	Multilayered spindle epithelium	Lens fiber swelling	Liquefaction/ vacuolar change	Calcification	Bizarre nuclei of lens fiber	Cataract index
A (baseline)	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}
B (AIN-93 diet)	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}
C (AIN-93 + 30% galactose)	$3.8 \pm 0.3^{\#}$	$3.9 \pm 0.2^{\#}$	$0.1 \pm 0.2^{*}$	$0.1 \pm 0.1^{*}$	$3.6 \pm 0.2^{\#}$	$2.9 \pm 0.3^{\#}$	$1.1 \pm 0.2^{\#}$	$15.5 \pm 0.8^{\#}$
D (AIN-93 + 30% galactose + 500 mg kg^{-1} extract)	$0.8 \pm 0.1^{\S}$	$0.3 \pm 0.1^{\S}$	$0.2 \pm 0.1^{*}$	$0.9 \pm 0.2^{\#}$	$1.7 \pm 0.2^{\S}$	$1.6 \pm 0.1^{\S}$	$1.2 \pm 0.1^{\#}$	$6.7 \pm 0.6^{\S}$
E (AIN-93 + 30% galactose + 1000 mg kg^{-1} extract)	$1.6 \pm 0.2^{\varphi}$	$0.3 \pm 0.1^{\S}$	$0.3 \pm 0.2^{*}$	$0.1 \pm 0.1^{*}$	$2.0 \pm 0.2^{\S}$	$1.7 \pm 0.2^{\S}$	$1.9 \pm 0.1^{\S}$	$7.9 \pm 0.6^{\S}$
B1 (B + AIN-93 + saline)	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}	0^{*}
C1 (C + AIN-93 + saline)	$3.2 \pm 0.3^{\#}$	$3.0 \pm 0.3^{\#}$	$0.2 \pm 0.1^{*}$	$0.2 \pm 0.1^{*}$	$2.3 \pm 0.2^{\S}$	$2.6 \pm 0.3^{\#}$	$0.1 \pm 0.1^{*}$	$11.6 \pm 0.6^{\varphi}$
C2 (C AIN-93 + extract)	$2.3 \pm 0.1^{\vartheta}$	$2.0 \pm 0.1^{\varphi}$	$0.1 \pm 0.1^{*}$	$0.1 \pm 0.1^{*}$	$1.9 \pm 0.2^{\S}$	$2.1 \pm 0.2^{\#\S}$	$0.1 \pm 0.1^{*}$	$8.6 \pm 0.6^{\S}$

Note: [a]Values are mean of 5 determinations ± SEM. [b]The *, $^{\vartheta}$, $^{\#}$, $^{\varphi}$, and § denote that value is significantly different ($p < 0.05$) from others in the same column but not different from value with a similar superscript.

Fig. 1. Photomicrograph of lens of rat before commencement of treatment (baseline, A) showing normal nuclei of lens epithelium (short arrows) and lens fibers (quad arrows). No TUNEL positive cells were observed.

treatment (A) neither was any lenticular damages observed in these two groups.

Figs. 1–7 show the result of the gross histological findings after four (4) weeks study period. The lens of rats placed on high dietary galactose (B) was observed to be swollen, degenerated vacuolated, liquefied and showed some atypical nuclei flat epithelial and macrophages in the vitreous cavity and between degenerated lens fibers. Although the lenticular damage was severe, no corneal or retinal damage was seen. No significant alteration in the cuboidal epithelium and lens fiber was observed in the lens of the control rat and those of the ones treated with the extract, except that an increased presence of macrophages was observed in the lenses of rats placed on dietary galactose and simultaneously treated with the extract. At the end of two weeks following withdrawal of dietary galactose, the epithelium of the normal control rat was observed to be intact while that of the rat previously placed on dietary galactose and treated with normal saline was observed to be swollen, degenerated vacuolated and showed signs of calcification. However, the observed damages were not noticed to be severe compared with the lens photomicograph prior to withdrawal of the dietary galactose. The photomicograph of the lens of rats treated with the extract simultaneously with the withdrawal of dietary galactose show a greater sign of recovery from damage caused to the lens by the dietary galactose.

Fig. 2. Photomicrograph of lens of rat placed on AIN-93 (galactose free) diet (normal control, B) showing normal nuclei of lens epithelium (short arrows) and lens fibers (quad arrows). No TUNEL positive cells were observed.

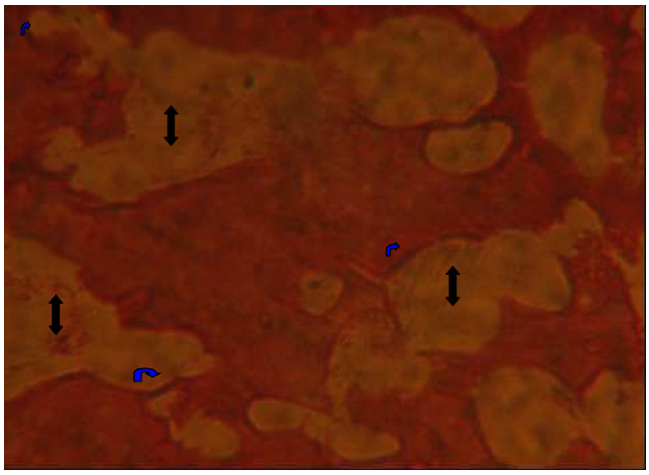

Fig. 3. Photomicrograph of lens of rats placed on 30% dietary galactose (test control, C) showing vacuolations (up–down arrows), liquefaction and atypical nuclei flat epithelium (bent arrows).

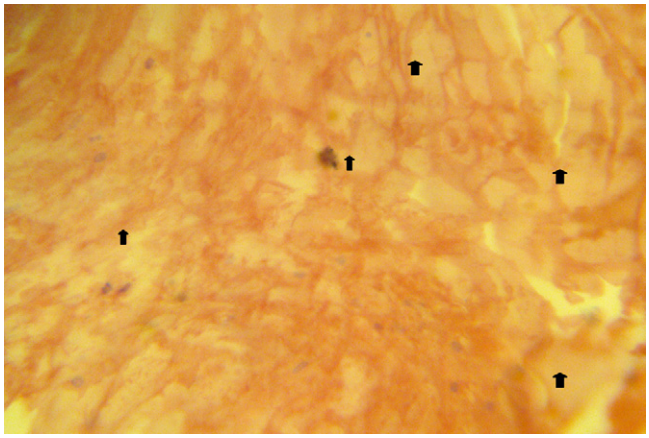

Fig. 4. Photomicrograph of rats placed on 30% galactose diet and simultaneously treated with 500 mg kg^{-1} (D) extract (D) showing patches of calcification (up arrows).

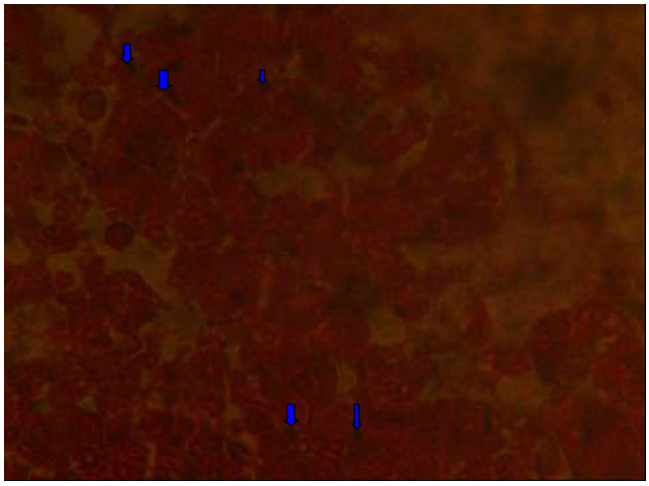

Fig. 5. Photomicrograph of lens of rats placed on 30% dietary galactose and treated with 1000 mg kg^{-1} extracts (E) showing disoriented lens fibers and Sparse TUNEL positive cells identified (down arrow).

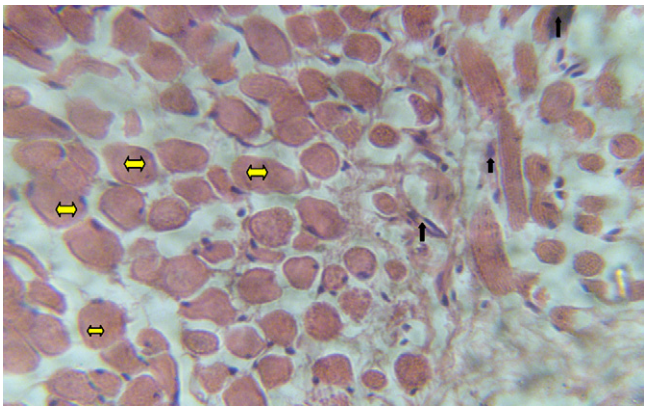

Fig. 6. Photomicrograph of lens of rats treated with the extract simultaneously with the withdrawal of dietary galactose (C2) showing lens fibers (right–left arrows) and nuclei of macrophages (up arrows). Sparse TUNEL positive cells identified.

3.2. Lens peroxidation and antioxidants status

Data obtained from the study indicates that high dietary galactose caused significant increase in lens peroxidation (Table 2) as the thiobarbituric reactive substance (TBARS) observed for lens of rats placed on dietary galactose was significantly higher than that obtained for the normal control and the baseline value. Withdrawal of dietary galactose after the fourth week further reduced the lens TBARS as determined at the end of the sixth week significantly. Maintenance of rats on dietary galactose along with simultaneous administration of the extract at all the two tested doses prevented the increase in TBARS significantly. The values obtained at 500 mg kg^{-1} and 1000 mg kg^{-1} dose were however not different from each other. They were also not significantly different from the observed values in the normal control and that of the baseline. When dietary galactose was withdrawn and the animals were simultaneously treated with the extract, the observed TBARS was not significantly different from the normal cond at the end of the sixth week significantly. Maintenance of rats on dietary galactose along with simultaneous administration of the extract at all the two tested doses prevented the increase in TBARS significantly. The values obtained at 500 mg kg^{-1} and 1000 mg kg^{-1} dose were however not different from each other. They were also not significantly different from the observed values in the normal control and that of the baseline. When dietary galactose was withdrawn and the

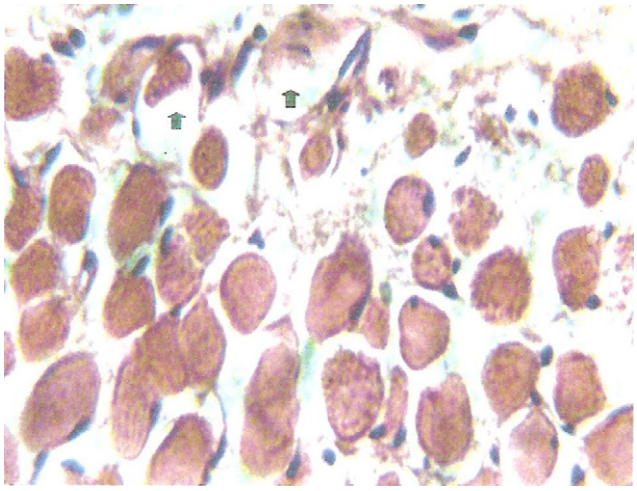

Fig. 7. Photomicrograph of lens of rats after withdrawal of dietary galactose (C1) showing vacuolations (up arrows) and macrophages. TUNEL positive cells were identified.

Table 2
Level of lens peroxidation and antioxidant status of rats after treatment.

Group/treatment	TBARS ($\times 10^5$ nmol MDA/g lens)	GSH ($\times 10^3$ μg/g lens)	Catalase ($\times 10^3$ μg/mg protein)	SOD ($\times 10^3$ ng/mg protein)
A (baseline)	1.42 ± 0.28*	6.99 ± 0.07*§	3.15 ± 0.23*	1.42 ± 0.22*
B (AIN-93 diet)	1.47 ± 0.15*	7.10 ± 0.13*§	3.28 ± 0.35*	1.39 ± 0.12*
C (AIN-93 + 30% galactose)	2.24 ± 0.13#	6.31 ± 0.12#	2.65 ± 0.09#	0.64 ± 0.31#
D (AIN-93 + 30% galactose + 500 mg kg^{-1} extract)	1.77 ± 0.22*¢	7.18 ± 0.03*	3.19 ± 0.07*	1.61 ± 0.31*
E (AIN-93 + 30% galactose + 1000 mg kg^{-1} extract)	1.65 ± 0.28*¢	6.67 ± 0.23*#¢§	2.25 ± 0.07¢	1.13 ± 0.23*#
B1 (B + AIN-93 + saline)	1.58 ± 0.10*	6.94 ± 0.21*¢§	3.22 ± 0.21*	1.41 ± 0.10*
C1 (C + AIN-93 + saline)	1.90 ± 0.12¢	6.28 ± 0.20#¢	2.32 ± 0.11¢	0.61 ± 0.10#
C2 (C + AIN-93 + extract)	1.49 ± 0.06*	6.79 ± 0.11§	2.88 ± 0.13*#	0.99 ± 0.12*#

Note: [a]Values are mean of 5 determinations ± SEM. [b]The *, #, and ¢ denote that value is significantly different ($p < 0.05$) from others in the same column but not different from value with a similar superscript.

animals were simultaneously treated with the extract, the observed TBARS was not significantly different from the normal control value but was significantly lowered compared with that obtained for the group that was treated with normal saline, though placed on similar diet.

The result of the GSH status after treatment (Table 2), indicates that rats placed on dietary galactose show a significantly reduction in their lens reduced glutathione (GSH) level when compared with the normal control. The value observed for the rats placed on dietary galactose was also significantly lowered compared with the baseline value. Withdrawal of dietary galactose after the fourth week did not significantly alter the GSH level from the observed value prior to the withdrawal. Treatment with the extract simultaneously with the dietary galactose prevented reduction in GSH level in the lens. Withdrawal of dietary galactose and simultaneous treatment with the extract significantly raised the lens GSH above that of the group that was placed on normal saline and maintained on the same diet (C1). However, the observed GSH was not different from that of the normal control and the baseline value.

Lens catalase activity was observed to be significantly reduced from that of the normal control when rats were placed on dietary galactose (Table 2). Simultaneous treatment with the extract at 500 mg kg^{-1} dose of the extract prevented reduction in catalase activity. However, when compared with the group treated with 1000 mg kg^{-1} dose, the catalase activity was higher. Withdrawal of dietary galactose significantly reduced the catalase activity further as the observed activity was significantly lowered when compared with catalase activity prior to withdrawal of the dietary galactose (Group C). Though treatment of the rats with the extract simultaneously with the withdrawal of dietary galactose did not significantly alter the catalase activity when compared with observed activity when the rats were placed on dietary galactose diet, the activity was however than that recorded in the group of rat treated with normal saline and placed on the same diet.

Table 2 also indicates that there was a significant reduction in the superoxide dismutase activity when rats were placed on dietary galactose. However, the observed activity at the end of two weeks following withdrawal of dietary galactose (C1) was not different from the value observed prior to withdrawal of the diet (C). Treatment with the extract simultaneously with dietary galactose prevented variation in superoxide dismutase activity from that of the control and the baseline. The observed activity at 500 mg kg^{-1} dosage was higher when compared with that of the group of rat that was not treated but that was placed on dietary galactose. However, the superoxide dismutase activity at 1000 mg kg^{-1} dose was different from that of the group placed on dietary galactose but not treated with the extract. No significant difference was observed in the activity of the extract between the two treated doses. Following withdrawal of dietary galactose and subsequent treatment with the extract (C2), the superoxide dismutase activity was not significantly different from that of the group that was treated with normal saline (C1). However, the activity was also not significantly different when compared with that of the normal control and the baseline value.

3.3. Lens protein

As shown in Table 3, the result obtained for total protein in the study indicates that dietary galactose did not cause a significant change in lens total protein concentration. The result also indicates that simultaneous administration of the extract at all the tested doses did not cause a significant alteration in lens total protein concentration. Neither withdrawal of dietary galactose nor administration of the extract simultaneously with the withdrawal was observed in the study to have significantly altered the lens total protein concentration.

The result of the soluble protein contents of lens (Table 3) also indicates that dietary galactose significantly lowered soluble protein content of the lens. Simultaneous administration of the extract at 500 mg kg^{-1}, prevented reduction in lens soluble protein concentration as the soluble protein observed was not significantly different from that of the normal control group but higher than that of the untreated group. However at 1000 mg kg^{-1}, administration

Table 3
Effect of treatment on lens total protein, soluble protein and insoluble lens protein concentration.

Group/treatment	Protein(mg/g lens)			
	Total	Soluble	Insoluble	% soluble protein
A (baseline)	96.28 ± 0.19*	61.50 ± 0.20*	35.78 ± 0.25*	63.88
B (AIN-93 diet)	105.02 ± 0.33*	65.12 ± 0.03*	37.90 ± 0.56*	62.01
C (AIN-93 + 30% galactose)	99.44 ± 0.36*	48.34 ± 0.40#	46.26 ± 0.50#	48.61
D (AIN-93 + 30% galactose + 500 mg kg^{-1} extract)	100.74 ± 1.41*	66.06 ± 0.20*	36.68 ± 0.66*	65.57
E (AIN-93 + 30% galactose + 1000 mg kg^{-1} extract)	98.22 ± 0.38*	49.72 ± 0.64#	41.50 ± 0.56¢	50.62
B1 (B + AIN-93 + saline)	106.00 ± 5.13*	65.01 ± 0.44*	38.99 ± 0.21*	61.33
C1 (C AIN-93 + saline)	100.10 ± 0.89*	50.21 ± 0.16#	47.65 ± 0.14#	50.16
C2 (C + AIN-93 + extract)	97.68 ± 4.16*	56.21 ± 0.21¢	43.51 ± 0.13§	56.52

Note: [a]Values are mean of 5 determinations ± SEM. [b]The *, #, § and ¢ denote that value is significantly different ($p < 0.05$) from others in the same column but not different from value with a similar superscript.

the lens soluble protein concentration was not significantly different from that of the untreated group (C) but lower than that of the normal control and the baseline. The lens soluble protein concentration of rats treated with 500 mg kg^{-1} dose of the extract was found to be significantly higher than that of the group treated with 1000 mg kg^{-1}. Withdrawal of dietary galactose did not significantly alter the lens soluble protein concentration when compared with the observed value prior to the withdrawal. Treatment with the extract simultaneously with the withdrawal of dietary galactose, however, raised the lens soluble protein concentration above the value observed for the group of rat placed on normal saline.

Table 3 also indicates that the lens insoluble protein concentration increased significantly with dietary galactose treatment. Simultaneous administration of the extract at 500 mg kg^{-1} was observed to prevent increase in lens insoluble protein concentration. Though the observed lens insoluble protein concentration at 1000 mg kg^{-1} was observed to be lower than the value observed in the group not treated, the observed value was significantly higher when compared with the normal control and the baseline value. Withdrawal of dietary galactose and simultaneous treatment with the extract C2 significantly lowered the lens insoluble protein concentration below the one obtained for rats treated with normal saline (Group C1). The observed lens insoluble protein concentration was however higher when compared with the normal control and the baseline concentrations. The lens insoluble protein concentration of the control rats was not different after sixth week of treatment B1 when compared with the value at the end of the fourth week (B).

A percentage soluble protein of 62.01 was observed for the normal control rat and this was reduced to 48.61 when the rats were placed on dietary galactose. Treatment with the extract at 500 mg kg^{-1} and 1000 mg kg^{-1}, however, raised the percentage soluble protein to 65.57 and 50.62 respectively. Whereas the percentage soluble protein observed with withdrawal of dietary galactose was 50.16, this was raised to 56.52 when the extract was introduced simultaneously along with the withdrawal of dietary galactose.

4. Discussion

Previous study has reported the use of 30% dietary galactose in inducing cataract and galactose-induced cataract is well accepted as an animal model for an investigation of the cataracts in human (Suryanarayana et al., 2003; Ai et al., 2000). The present study is consistent with these previous findings. An important finding from this study is that *Hydrocotyl bonariensis* leave extract offers protection when used prior to the onset of cataract. We also report here that this protective ability is dose dependent and that it is best achieved at low dose.

Different agents are known to have different mechanisms for cataract formation (Gehring, 1971). Methyl nitrosourea (MNU) for example causes cataract by producing DNA adducts in the lens epithelial cells, leading to suppression of DNA synthesis followed by apoptosis, which peaks three (3) days after the MNU administration (Yoshizawa et al., 2000). Enhanced proliferation of the lens epithelial cells has been recognized in several types of cataracts (Fagerholm, 1982; Kubo et al., 1999; Majima et al., 1998). The present study indicates that dietary galactose, induces proliferation of lens epithelium, lens vacuolation, liquefaction of fibers and macrophages. Although there are many reports on induction and inhibition of cataract, the relevant molecular and cellular mechanisms are still unclear (Huang et al., 2007). Abnormal lens epithelial proliferation has been implicated in the acceleration of cataract formation (Katsuji et al., 2002). Macrophages within the lens may be due to a reaction that targets the damaged lens component (Zimmermann, 1964). These macrophages may be responsible for cell debris removal or contrarily, may be capable of damaging lenses by releasing numerous potential mediators (Zigler et al., 1983). Data generated on the cataract index and the result of the histological damage suggests the possibility that *Hydrocotyl bonariensis* leave may reduce apoptosis in galactose-induced cataract. Intriguingly, lens epithelial apoptosis has been implicated as a common cause of non-congenital cataracts (Yoshizawa et al., 2000). A typical lens undergoes a series of changes during the cataractous process. The first event that occurs is the separation and liquefaction of the anterior suture. After this, the separated part of the anterior suture gradually becomes covered by proliferative epithelial cells. As a consequence, lens fiber cells regenerate leading to the recovery of lens transparency. However, upon completion of the repair, this proliferative epithelial cells gradually decrease in numbers and becomes vacuolated and swollen so that the recovery of the cataract is halted (Masayuki et al., 2006).

It may be important to emphasize that though cataract index was found to have reduced with the arrest of hypergalactosemia, and simultaneous administration of the extract, the rats lens may still be cataractous. In a previous study by Katsuji et al. (2002) with N-methyl-N-nitrosourea, a matured cataract was reported with a cataract index of 0.3. Our study thus suggest that arrest of hypergalactosemia after cataract onset may not reverse cataractogenesis and also that administration of the extract at this point reduces cataract progression.

There is substantial evidence that oxidative stress is at least partly responsible for galactosemic cataract and that antioxidants can be effective inhibitors of cataractogenesis in this model (Saxena et al., 1996; Suryanarayana et al., 2003). Three possible mechanisms that have been proposed for the formation of cataract resulting from hyperglycemia and hypergalactosemia are the polyol pathway, oxidation and non-enzymatic glycation (Spector, 1991). In this study, we have investigated the effect of *Hydrocotyl bonariensis* administration on lens oxidation and lens protein concentration. Generally, lipid peroxidation is assessed by determination of thiobarbituric acid reacting substances (TBARS) particularly malonaldehyde (MDA). MDA is formed by cleavage of each side of endoperioxide ring. MDA introduces cross-link in proteins which may induce profound alteration in their biochemical properties (Varshney and Kale, 1990). An increased MDA value observed in our study along with a decrease in the measured antioxidant status is consistent with previous reports (Suryanarayana et al., 2003) of increased oxidative stress induced by dietary galactose. A consequent increase in GSH level, catalase and SOD activities as reported in this study, suggest the efficacy of the extract in reducing lens peroxidation and which may support its usage as an anticataractogenic agent and that the efficiency may be better achieved at dosage of about 500 mg kg^{-1}.

Data from the remediation study suggest that withdrawal of galactose moderately decreased the thiobarbituric acid reducing substance but did not boost the endogenous antioxidants status suggesting that there continues to be increased oxidative stress following the arrest of hypergalactosemia. Subsequent administration of the extract at this point seems to lower the degree of peroxidation and does not reduce the endogenous antioxidant status further, suggesting that though administration of the extract within the period of our study does not completely reverse cataractogenesis, it does reduce the progression.

The result obtained for total protein in the present study indicates that in the galactose model of experimental cataract, there is no significant change in lens total protein concentration. This differs from the observation of Kyselova et al. (2005) which reported a decrease in lens total protein with streptozocin induced diabetic cataract but agrees with that of Steven et al. (2005) which reported no significant variation in lens total protein of human cataractous lens but a reduction in soluble protein. Data from this study

thus suggests the efficacy of the extract in reducing aggregation of lens protein and consequently prevention of cataract induction at a lower dose but not at dosage of 1000 mg kg^{-1}. Though the principle for significant reduction in severity of cataract by the extract at lowered dose when compared with a higher dose is not very clear as of now, our result is comparable to a study reported by Suryanarayana et al. (2003) where the efficacy of curcumin on galactose-induced cataractogenesis was investigated. Their reports indicated that whereas decrease in lens protein was prevented by 0.002% curcumin, this was not noticed with 0.01% and that with a 10-fold increase in the dosage (0.02%), there was an enhanced cataract progression when compared to galactose-induced cataract. Their report also indicated that both the total protein and soluble protein were reduced with cataract development and that both were significantly reduced with 0.02% curcumin (when compared with the control group and the untreated group). It is important however to note as reported in the present study that increased dosage of the extract does not indicate an enhanced progression of cataract.

A reduction in percentage soluble protein from approximately 81.0% in adult human transparent lens to only 51.4% in cataractous lens has been reported (Steven et al., 2005). Another study in streptozocin induced cataract in rat reported a reduction in soluble protein from 72.5% to 46.9% (Suryanarayana et al., 2005). A percentage reduction from 71 to 60 with galactose-induced cataract has also been reported (Suryanarayana et al., 2003). In the present study, a reduction in percentage soluble protein from 62.01 in normal lens to 48.61 in cataractous rat lens is reported. We also report here that with the administration of *Hydrocotyl bonariensis* leave extract at 500 mg kg^{-1}, the percentage soluble protein increases to 65.57. Our findings also indicates that following withdrawal of galactose and subsequent administration of the extract the percentage soluble protein of 56.52 obtained was higher than the baseline value and also higher than 50.16 obtained for the group treated with normal saline. Although there is a battery of other posttranslational modifications, accumulation of large amounts of insoluble protein derived from the otherwise soluble protein due to aggregation has been identified as the major biochemical mechanism in cataractogenesis (Reddy et al., 2002). Our findings in this study thus suggest that *Hydrocotyl bonariensis* extract may prevent the conversion of soluble protein to insoluble protein and hence may reduce protein aggregation. Finding from the remediation study also suggests that withdrawal of galactose does not reduce the insoluble protein concentration whereas a moderate reduction in the concentration of the insoluble protein was achieved with the administration of the extract. Since total lens protein concentration was not observed to change with dietary galactose treatment, but that a variation only occurred with soluble and insoluble protein, we opined that cataract only affects protein solubilization. Our study also suggests that administration of the extract prior to cataract induction and after cataract onset did not affect total protein but only prevents lens protein insolubilization.

5. Conclusion

Our study has demonstrated that *Hydrocotyl bonariensis* leave extract offers protection from cataract induction by reducing lens protein insolubilization and lens peroxidation and by increasing lens antioxidant status. Consequently, a reduction in lens apoptosis and epithelial proliferation occurred. The study also reports that administration of the extract after the onset of cataract may reduce cataract progression.

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