

The effect of *Cucumis sativus* L. and *Cucumbita pepo* L. (Cucurbitaceae) aqueous preparations on galactose-induced cataract in Sprague-Dawley rats

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In Ghana cataract is said to be the leading cause of blindness. However, management through surgery is accompanied by several adverse effects and takes care of only about a quarter of the need. The study therefore was aimed at investigating claims that *Cucumis sativus* and *Cucumbita pepo* have anti-cataract properties. Prior to induction of cataract in Sprague-Dawley rats using galactose and through the cataract induction period, groups of rats were treated with 50, 100, and 150 mg/kg of preparations of *C. sativus* or *C. pepo*, *per os*, daily and compared with controls. An acute and delayed toxicity study was also performed on the herbal products. The galactose only treated group showed consistently significant ($P \leq 0.001$) development of cataract over the 4-week study period. The *Cucumis sativus* and *Cucumbita pepo* preparations steadily and significantly ($P \leq 0.001$) delayed cataract formation over the period, with *Cucumbita pepo* performing better. The cataract scores recorded for the combined treatment with the herbal preparations and galactose were significantly lower ($P \leq 0.001$) compared to galactose only treated group. Doses below 150 mg/kg ingested did not show acute toxic symptoms and mortality. This study therefore has established that *Cucumis sativus* and *Cucumbita pepo* when used in lower doses regularly could be effective in delaying cataractogenesis. The No-Observable-Adverse-Effect level (NOAEL) was 50-150 mg/kg; LD₅₀ was greater than 250 mg/kg.

Keywords: *C. sativus*, *C. pepo*, Galactose-Induced Cataract, Antioxidant activity.

INTRODUCTION

Cataract, a disease of the eye, is the clouding of the clear, natural lens located in the eye which causes it to lose its transparency and hence not allow sufficient light to reach the retina, thus impairing vision (Truscott, 2000). The WHO describes cataract as the leading cause of blindness (51 %) in the world: represents about 20 million people (WHO, 2010). The majority of those blind live in developing countries. Globally, at least 100 million eyes have visual acuity <6/60 due to cataract (Foster, 1999). In Africa and in Ghana, cataract is said to be the leading cause of blindness. It has been reported that about

46,000 persons suffer from blindness annually in Ghana and only 16,000 of them are treated with the rest becoming unproductive after becoming blind (Debrah, 2010).

Even though surgical intervention is available for treatment, cataract surgery may come with adverse effects such as corneal edema, refractive errors, post-capsular opacity, flat anterior chamber with increased intra-ocular pressure, dislocated intra-ocular lens, anterior uveitis, hyphema, subconjunctival haemorrhage, and wound leak with decreased intra-ocular pressure. Owing to these adverse effects, people are hesitant to undergo the surgical procedure in resolving cataract. In Ghana, cataract surgery is very expensive and unaffordable for the majority of patients. Even though cataract surgery is included in the National Health

Insurance Scheme, the problem of lack of human resource and infrastructure leads to poor accessibility of intervention for people in the rural areas. That notwithstanding, the total number of surgeries performed currently take care of approximately a quarter of the need (GEF, 2010).

There is therefore an urgent need to find alternatives of cataract treatment/management which may be safer, affordable, and acceptable by a majority of the population. Current trends involve developing natural products with the notion that it is safer and affordable (Gurib-Fakim, 2006).

A number of herbal practitioners and traditional healers in Ghana who claim they have herbal preparations that can prevent, delay, or treat cataract. Individuals who fear the consequences of cataract surgery and those looking for affordable alternatives resort to the use of these herbal preparations. Two plants commonly used in these preparations are *Cucumis sativus* and *Cucumbita pepo*.

Cucumis sativus, commonly called cucumber, has been used in traditional medicine in several ailments. The leaf juice is emetic, it is used to treat dyspepsia in children (Duke and Ayensu, 1985). The fruit is depurative, diuretic, emollient, purgative and resolvent (Chiej, 1984; Duke and Ayensu, 1985). The fresh fruit is used internally in the treatment of blemished skin, heat rash etc., whilst it is used externally as a poultice for burns, sores etc and also as a cosmetic for softening the skin (Duke and Ayensu, 1985; Bown, 1995). The seed is cooling, diuretic, tonic and vermifuge (Grieve, 1971; Duke and Ayensu, 1985). Thoroughly ground seeds (including the seed coat), is a standard dose as a vermifuge and usually needs to be followed by a purgative to expel the worms from the body (Grieve, 1971). A decoction of the root is diuretic (Duke and Ayensu, 1985). Studies on *Cucumbita pepo*, commonly called pumpkin, have shown that the seed oil is potent at relieving chronic rheumatoid arthritis. It reduces symptoms of Benign Prostatic Hyperplasia. It has acute schistosomiasis, and helps prevent the most common type of kidney stone (Rybalovskii, 1966; Harvath and Bedo 1988; Carbin and Eliasson, 1989; Eagles, 1990; Fahim *et al.*, 1995).

The study was aimed at investigating the effect of *Cucumis sativus* and *Cucumbita pepo* on galactose-induced cataract in Sprague-Dawley rat and to ascertain its safety for use.

MATERIALS AND METHODS

Preparation of the Herbal Products and Dosing

Prepackage decoctions made from the whole plant, of *Cucumis sativus* or *Cucumbita pepo* prepared by the Herbalist, Habibi Herbal Clinic, Sepe Tinpom, Kumasi in the Ashanti Region of Ghana. A volume of 4 litres each of

the *Cucumis sativus* and *Cucumbita pepo* preparations was condensed under low temperature and pressure using a Buchi Rotor Evaporator (Rotavapor R-210, Switzerland) and dried in a Gallenkamp hot air oven (Oven 300 plus series, England) maintained at a 40°C (for 24 hours) to yield 25 g and 19 g respectively. The dried *C. sativus* and *C. pepo* products were reconstituted in distilled water for use in this study and would be referred to as HP1 and HP2 respectively.

Dosing of HP1 and HP2

Dosing of HP1 and HP2 in this study was based on manufacturer recommendation which was calculated to be 50 mg/kg/day. Dosing was a single event at a volume of 10 ml/kg body weight. Doses used were calculated based on manufacturer's recommendation. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used because it is the intended human exposure route.

Animals and Husbandry

Male Sprague-Dawley rats, 3 weeks of age, obtained from the Animal Facility of Department of Bioscience, KNUST, Ghana, were allowed to acclimatize for one week prior to initiation of dosing. During this period, rats were observed (physical; in-life) daily and weighed. At initiation of treatment, animals were approximately 4 weeks old. Individual weights of rats placed on test were within $\pm 15\%$ of the mean weight. All rats were examined during the acclimatization period to confirm suitability for study. Animals were housed in stainless steel, wire mesh cages during the acclimation and the experimental periods. The rats were kept under ambient light/dark cycle, room temperature and relative humidity. The animal had free access to pelleted rat chow (GAFCO, Tema, Ghana) and water daily.

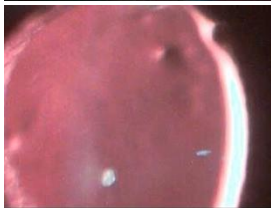
Drugs and Chemicals used

Galactose (BDH, Poole, England) was used to induce cataract. A 1% Tropicamide eye drop (Egyptian Int Pharmaceuticals Industries Co., Egypt) was used to dilate the pupil of the eye at every slit lamp examination.

Induction of Cataract

Rats were given 0.5 ml of 50% galactose daily. The lenses in the anterior segment of the eye were observed weekly (after pupillary dilation with 1% tropicamide) using

Table 1. The grade, description, score, and slit lamp photographs for the various grades of galactose-induced developing cataracts in Sprague-Dawley rats.

Grade	Description	Cataract score	Photograph of Description
0	Clear lens with no vacuole	0	
1	clear lens with less than 3 vacuoles	1	
2	Clear lens more than 3 vacuoles	2	
3	vacuoles covering the entire surface of the lens	3	
4	Partial lens opacity	4	
5	Total lens opacity	5	

a SL500 Shin Nippon Slit Lamp (Ajinomoto Trading Inc., Tokyo, Japan) fitted with a digital microscope camera (Olympus, Tokyo, Japan) over a 4-week period. Cataract formation, seen as the appearance of vacuoles, and partial or total opacification of one lens, was sought for with the help of an eye care professional. Digital photographs of the lenses were taken and the various stages of cataract formation were established and graded.

Grading and Scoring of Cataract

The grading of cataract was developed by the researchers after several observations in preliminary tests of inducing cataract with galactose in Sprague-Dawley rats for the purpose of this study. The cataract score for the various groups (controls and treatment) was estimated from the grading obtained upon comparison of the appearance of the lens with that in the Table 1 above.

Table 2. The grade of cataract formed for the controls, PH1, and PH2-treated Sprague-Dawley rats from week 1 to week 4.

Groups	Grade of Cataract Formed			
	Week 1	Week 2	Week 3	Week 4
I: no treatment (control 1)	0	0	0	0
II: 100 mg/kg PH1 (control 2)	0	0	0	0
III: 100 mg/kg PH2 (control 3)	0	0	0	0
IV: GAL	1	3	5	5
V: 50 mg/kg PH1 + GAL	1	2	3	3
VI: 100 mg/kg PH1 + GAL	0	2	3	3
VII: 150 mg/kg PH1 + GAL	0	2	3	3
VIII: 50 mg/kg PH2 + GAL	0	1	2	2
IX: 100 mg/kg PH2 + GAL	0	1	2	2
X: 150 mg/kg PH2 + GAL	0	1	2	2

GAL= Galactose

Effect of PH1 and PH2 on Cataract Formation

Sprague-Dawley rats were grouped into ten (I-X) with five per group. Group I (control 1) was just kept under experimental conditions. Groups II (control 2) and III (control 3) were treated with 100 mg/kg PH1 and 100 mg/kg PH2 respectively through the experimental period. Group IV was treated with 50% galactose only. Groups V, VI, and VII were treated with 50, 100, or 150 mg/kg of PH1, 7 days prior to cataract induction, followed by the same treatment plus 50% galactose through the experimental period. Groups VIII, IX and X were treated with 50, 100, or 150 mg/kg PH2, 7 days prior to cataract induction, followed by the same treatment plus 50% galactose through the experimental period. Lenses in the anterior chamber of the eyes were observed weekly for 4 weeks for signs of cataract formation of each animal in the various groups. The total cataract score for each group per week were recorded and compared statistically for variation.

Acute and Delayed Toxicity Test

The rats were assigned to treatment groups 1-6, with five (5) in a group. Group 6 was the non-drug treatment group (control). PH1 was administered at doses of 50, 100, 150, 250, and 500 mg/kg (representing the stated daily dose, two, three, five and ten times the daily dose respectively). Observation for clinical and behavioral symptoms of toxicity and mortality were made by filming for the first 60 minutes after which observations were made hourly for 24 hours. Symptoms of delayed toxicity were sought daily for 14 days. The time of onset, intensity, and duration of these symptoms, if any, was recorded. The same procedure was performed for PH2.

Ethical Consideration

This study was conducted in compliance with all appropriate parts of the Animal Welfare Act Regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112–36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426–6505 effective March 18, 1991.

Statistical Analysis

The statistical analysis of data obtained were made using GraphPad Prism Version 5.0 [GraphPad Software, Inc. USA]. Differences between treatment groups and the controls were estimated using One-way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test (post-hoc test) at a confidence level of 95 %. Probability values less than or equal to 5 % ($P \leq 0.05$) were considered significant.

RESULTS

Effect of HP1 and HP2 on Galactose-induced Cataract formation

Results obtained indicated that controls 1, 2, and 3 had not cataract formation (Grade 0; Cataract score 0 ± 0.00) over the 4-week study period (Table 2 and 3). The galactose only treated group showed consistently significant ($P \leq 0.001$) development of cataract (clear lens with less than 3 vacuoles through to total lens opacity in both eyes) over the study period.

PH1 and PH2 steadily and significantly ($P \leq 0.001$) delayed cataract formation with PH2 performing better

Table 3. The cataract scores for the controls, PH1, and PH2-treated Sprague-Dawley rats from week 1 to week 4 after cataract induction with galactose.

Groups	Cataract Score			
	Week 1	Week 2	Week 3	Week 4
I: no treatment (control 1)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
II: 100 mg/kg PH1 (control 2)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
III: 100 mg/kg PH2 (control 3)	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
IV: GAL	0.8 ± 0.44 ***	3.0 ± 0.00 ***	5.0 ± 0.00 ***	5.0 ± 0.00 ***
V: 50 mg/kg PH1 + GAL	0.8 ± 0.44 ***, ns	2.0 ± 0.70 ***, ††	3.2 ± 0.45 ***, †††	3.4 ± 0.45 ***, †††
VI: 100 mg/kg PH1 + GAL	0 ± 0.00 ns, †††	2.2 ± 0.45 ***, †	3.0 ± 0.00 ***, †††	3.0 ± 0.00 ***, †††
VII: 150 mg/kg PH1 + GAL	0 ± 0.00 ns, †††	2.0 ± 0.00 ***, ††	2.8 ± 0.45 ***, †††	3.0 ± 0.00 ***, †††
VIII: 50 mg/kg PH2 + GAL	0 ± 0.00 ns, †††	1.4 ± 0.54 ***, †††	2.0 ± 0.70 ***, †††	2.2 ± 0.45 ***, †††
IX: 100 mg/kg PH2 + GAL	0 ± 0.00 ns, †††	1.2 ± 0.45 ***, †††	2.2 ± 0.45 ***, †††	1.8 ± 0.45 ***, †††
X: 150 mg/kg PH2 + GAL	0 ± 0.00 ns, †††	1.0 ± 0.00 ***, †††	2.0 ± 0.00 ***, †††	2.0 ± 0.00 ***, †††

Values are Means ± SD (n=5).

- ns $P > 0.05$; *** $P \leq 0.001$ are the levels of significance between the controls and the other treatment groups determined using One way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test.
- ns $P > 0.05$; † $P \leq 0.05$; †† $P \leq 0.01$; ††† $P \leq 0.001$ are the levels of significance between the galactose only treated group and the galactose and PH1, and galactose and PH2 treated groups determined using One way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test.
- GAL= Galactose

than PH1. The highest grade obtained (after 4 weeks) for PH2-treated rats in which cataract were being induced was Grade 2 while that for PH1 was Grade 3 (Table 2). The cataract scores recorded for groups with combined treatment with PH1 and PH2 and galactose were significantly lower ($P \leq 0.001$) in the 2nd, 3rd and 4th weeks compared to galactose only treated group (Table 3).

Acute and Delayed toxicity test

After treatment with PH1 and PH2, no deaths were recorded in rats treated with less than 150 mg/kg over the entire experimental period (14 days). There was no lacrimation, salivation, urination labored breathing, constipation, emaciation, skin eruptions, abnormal posture, hemorrhage, sedation, diarrhoea, polyuria, polydipsia, polyphagia, anorexia, rhinorrhoea/nasal congestion, loss of autonomic reflexes, neuromuscular inco-ordination and collapse, hyperesthesia, hypothermia, twitching, spasticity, convulsion, writhing, tremors, fasciculations and respiratory depression. Observations of gait did not show, staggering, wobbly gait, hind limbs exaggerated, overcompensating, and/or splayed movements, feet (primarily hind feet) point outward from body, forelimbs dragging and/or showing abnormal positioning, nor walking on toes (the heels of the hind feet are perpendicular to the surface). Rats treated with 250 and 500 mg/kg showed a hunched, or raised up back, lethargy, piloerection, and loss of hair, and death of more than 50 % of the animals (in the 500 mg/kg treated group). The No-Observable-Adverse-Effect

level (NOAEL) was between 50 and 150 mg/kg for both PH1 and PH2.

DISCUSSION

In this study, it was evident that an excess of galactose in diet could result in cataract formation. Accumulation of high levels of sugar alcohol such as galactose causes hypertonicity of lens. Increased hypertonicity eventually increases sodium levels and decreases potassium levels leading to cataract formation (Newell, 1992). Animal studies clearly indicate that the rate and severity of sugar cataract development is proportional to both the lenticular levels of aldose reductase and the severity of the hyperglycemia (Lee, Chung 1999). The grade of cataract increased with time due to increased disruption of the lens metabolism caused by galactose.

The controls (1, 2, and 3) recording no cataract formation indicates that cataract formation seen in the other treatment groups is not associated with laboratory environmental conditions or PH1 and PH2 treatment.

HP2 effect of delaying the onset of cataract formation was significantly better than HP1. HP 2 was a product made from *Cucumbita pepo* which contains higher level of carotenoids than PH1 prepared from *Cucumis sativus*. Carotenoids are antioxidants and higher levels are associated with decreased risk for cataract (Bates *et al.*, 1996). The young lens has substantial reserves of antioxidants (e.g., vitamins C and E, carotenoids, and glutathione — GSH) and antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione

reductase/peroxidase) that may prevent damage. Therefore, these extracts are expected to prevent cataract formation in galactose-induced rats. *Curcubita pepo* is a good source of vitamin A, flavonoid polyphenolic antioxidants like leutin, xanthins and carotenes as well as vitamin C. The roles of flavonoid polyphenolic antioxidants in cataracts have been considerably less studied (Varma *et al.*, 2003), but various animal studies using various cataract models have demonstrated the effectiveness of specific nutrients in the prevention of cataracts. An early study showed that a full team of vitamins prevented and regressed galactose cataracts in rats. Vitamin C was found to be effective in preventing galactose cataracts in guinea pigs (Vinson *et al.*, 1992). An *in vivo* study confirmed, ascorbate appears to slow down but not prevent cataract formation in the galactose fed rats (Vinson *et al.*, 1992).

The antioxidant properties of the extract administered at daily dosage may be thought to have maintained the lens physiology by increasing intracellular vitamin or antioxidant levels, resulting in a slight delay of the onset of cataract formation was slightly delayed. In recent years, a great emphasis has been laid on exploring the possibility of using our natural resources to delay the onset and progression of cataract. A great number of medicinal plants and their formulations are reported to possess antioxidant properties and offer protection against cataract. Carotenoids are natural lipid-soluble antioxidants. It is reported that persons with a high intake of carotenes reduce the incidence of risk of cataract and the relationship between nuclear cataract and intakes of α -carotene, β -carotene, lutein, lycopene and cryptoxanthin stratifying by gender and by regular multivitamin use (Mares-Perlman, 1994; Brady *et al.*). Amongst all carotenoids lycopene has a high antioxidative activity and exerts a protective effect in various diseases. (Srivastava *et al.*, 2002). In previous studies, it was found that lycopene protects against oxidative stress-induced experimental cataract and prevented sugar-induced diabetic cataract. Curcumin, the active principle of turmeric, has been shown to have antioxidant activity Curcumin delayed the onset and maturation of galactose-induced and streptozotocin-induced diabetic cataracts (Suryanarayana *et al.*, 2003).

Although epidemiological studies and *in vitro* experiments suggest that antioxidants might protect the lens against cataract formation, most of these studies report inverse associations between the risk of cataract and at least one antioxidant nutrient — vitamin E (Jacques *et al.*, 2001).

The acute toxic symptoms observed with the higher doses of the preparation suggest that it should be used in doses suggested by the manufacturer. Doses that showed toxic symptoms were five to ten times the manufacturers stated dose. Lower doses are therefore recommended as these still show significant delays of cataractogenesis.

CONCLUSION

It has been established that *Cucumis sativus* and *Cucubita pepo* when used in lower doses regularly could be effective in delaying cataractogenesis. The No-Observable-Adverse-Effect level (NOAEL) was 50-150 mg/kg.

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