ACYMOPSIS TETRAGONOLOBUS AND CYPERUS ROTUNDUS PREVENTS OXIDATIVE STRESS IN DIABETIC CATARACT INDUCED WISTAR ALBINO RATS

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ABSTRACT

The present study was conducted to evaluate the effect of ethanolic extracts of Cyamopsis tetragonolobus pods and Cyperus rotundus rhizomes on the oxidative stress parameters of diabetic cataract induced Wistar albino rats. The experimental rats were grouped into 4: Group I – Normal controls, Group II – Diabetic cataract induced animals (streptozotocin-induced diabetic rats which developed cataract), Group III – Cyamopsis tetragonolobus extract co-treated animals and Group IV – Cyperus rotundus extract co-treated animals (animals were treated for 19 weeks with each of the plant extract after streptozotocin induction). Animals with diabetic cataract showed elevated levels of lipid peroxides and conjugated dienes, while Cyamopsis tetragonolobus and Cyperus rotundus co-treated groups showed lower levels. Enzymatic antioxidant (superoxide dismutase, catalase, glutathione peroxidase) levels decreased in rats with diabetic cataract while both the plant co-treated animals showed increased enzymatic antioxidant levels. Similarly, levels of non-enzymatic antioxidants such as reduced glutathione, vitamin C and vitamin E decreased in diabetic cataract rats, while both the plant treated animals showed increased levels. The above results thus, signify the antioxidative potential of both Cyamopsis tetragonolobus and Cyperus rotundus.

Keywords: Diabetic cataract, Cyperus rotundus, Cyamopsis tetragonolobus, Oxidative stress, Antioxidant.
INTRODUCTION
Diabetes mellitus is the most common non-communicable disease that occurs worldwide. Diabetes is a life-long disease and is considered as the second foremost reason to cause blindness and renal failure worldwide\(^1\). Diabetic cataract is a major complication of diabetes which affects most of the diabetic patients. Diabetic cataract is a clouding that develops in the crystalline lens of the eye or in its envelope. It is a visual impairment causing disturbance in lens transparency that is formed mainly due to opacification or optical dysfunction of crystalline lens\(^2\). Diabetes mellitus is profoundly connected to increased oxidative stress in diabetic patients and hyperglycemic animals\(^3\). Though, many mechanisms are related to hyperglycemia and diabetic complications, oxidative stress is equally involved in the pathogenesis\(^4\). Cataract surgery is the most commonly available treatment to treat diabetic cataract. Although this surgery regains vision, it has many drawbacks. Surgery will not resolve the vision problems completely\(^5\). Medicinal plants have been widely used in the treatment of various human diseases from time immemorial and are considered as alternative medicines employed for prevention of number of diseases\(^6\). Preventive measures with phytotherapy are gaining increasing importance these days. Even our daily food provides not only essential nutrients needed for life, but also other bioactive compounds for promoting health and to prevent diseases\(^7\).

*Cyperus rotundus* also known as nutgrass or coco-grass or purple nut sedge or red nut sedge is a species from the family Cyperaceae\(^8\). *Cyperus rotundus* was one of the common drugs in oriental folk medicine. This medicinal plant is reported to have numerous medicinal properties and is effective as antidyspeptic, aromatic, nervine tonic, diuretic, astringent and diaphoretic\(^9\). The guar bean or cluster bean (*Cyamopsis tetragonolobus*; family Fabaceae) is an annual legume and is the source of guar gum. *Cyamopsis tetragonolobus* have been used traditionally for various ailments\(^10\). This plant can be used as cooling agent, digestive tonic and galactagogue. They are used to treat conditions such as constipation, dyspepsia, anorexia, agalactia and vitated condition of kapha and pitta\(^11\).

In our present study, we have investigated the effect of the ethanolic extracts of *Cyamopsis tetragonolobus* pod and *Cyperus rotundus* rhizome on the oxidative stress parameters of diabetic cataract induced Wistar albino rats.
MATERIALS AND METHODS

(i) Plant Collection

*Cyamopsis tetragonolobus* pods (fruit) and *Cyperus rotundus* rhizomes were obtained from medicinal plant vendor in Chennai, Tamil Nadu, India without any external defects.

(ii) Authentication of Plants

The selected plant materials were identified and authenticated as PRAC/2010/495 for *Cyperus rotundus* and as PRAC/2010/494 for *Cyamopsis tetragonolobus* by botanist, Professor P. Jayaraman, Plant Anatomy Research Center, West Tambaram, Chennai, Tamil Nadu, India. The rhizomes and pods were shade dried at room temperature for one week and made into coarse powder.

(iii) Extraction of Plants

20gm coarse powder of *Cyperus rotundus* rhizomes and 20gm coarse powder of *Cyamopsis tetragonolobus* pods were homogenized in 100ml of 90% ethanol separately using Waring blender. The extraction was done as per the procedure followed in our earlier study[12]. The ethanolic extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

(iv) Animal Study

Eight week old healthy Wistar albino rats of either sex weighing 120±30gm were purchased from King Institute, Guindy, Chennai, Tamil Nadu, India. The animals were maintained at Saveetha University, Chennai, Tamil Nadu, India under standard conditions of humidity (45-55%), temperature (25±2°C) and light (12hr light/12hr dark) (IAEC No. Biochem BWC 010/10). They were fed with standard pelleted diet and given free access to water *ad libitum*. Experimental animals were handled according to the University and Institutional legislation, regulated by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

(v) Experimental Design

The experimental animals were divided into 4 groups as mentioned below:

Group I – 6 normal rats as controls; Group II – 6 diabetic cataract induced rats (diabetes was induced with streptozotocin(STZ) and maintained for 20 weeks to develop cataract; Group III – After STZ induction for a week, 6 rats were orally fed with 200mg/kg *Cyamopsis tetragonolobus* extract for 19 weeks; Group IV – After STZ induction for a week, 6 rats were orally fed with 200mg/kg *Cyperus rotundus* extract for 19 weeks.

The experimental design, grouping of animals and the induction of diabetic cataract are as described in our previous study[13].
(vi) Collection of Blood
All the animals were killed by cervical decapitation after the experimental period. The blood was collected without EDTA for the separation of serum.

(vii) Assessment of biochemical parameters in serum
The levels of conjugated dienes\(^{14}\) and lipid peroxides\(^{15}\) were measured in serum. Superoxide dismutase (SOD)\(^{16}\) and catalase (CAT)\(^{17}\) activities were estimated. Glutathione peroxidase (GPx) activity was measured\(^{18}\) with modifications\(^{19}\). The total reduced glutathione (GSH)\(^{20}\), ascorbic acid\(^{21}\) and vitamin E content\(^{22}\) were estimated in serum.

(viii) Statistical Analysis
Statistical analysis was done by using SPSS 16.0.1. All results were presented as mean value ± standard deviation (SD) for six samples in each group. One-way analysis of variance (ANOVA) with Tukey’s post test was used for statistical analysis of collected data. Differences were considered significant at P < 0.05.

RESULTS
1. Cataract Incidence
Cataract was observed after 20 weeks of STZ administration and verified by slit-lamp biomicroscope. STZ-administered rats alone showed the gradual progression of diabetes-induced cataract and the cataract was graded as Cortical Cataract (Fig.1).

Fig. 1 – Eye and Lens of experimental animals
The animals co-treated with the ethanolic extract of *Cyamopsis tetragonolobus* pod (Group III) and *Cyperus rotundus* rhizome (Group IV) for 19 weeks after STZ administration (after 1 week) did not show the development of cataract. Administration of both the extracts does not exert any toxic symptoms or side effects implying its non-toxicity. No death was observed during the study.

2. Oxidative stress in Serum

Table 1 shows the levels of lipid peroxides and conjugated dienes in the serum of experimental animals. Group II animals showed significantly elevated (p<0.001) levels of lipid peroxides and conjugated dienes as compared to Group I animals. *Cyperus rotundus* treated (Group IV) and *Cyamopsis tetragonolobus* treated (Group III) animals showed significantly lower (p<0.001) levels of lipid peroxides and conjugated dienes when compared to that of Group II animals. *Cyperus rotundus* treated animals showed near normal levels of serum lipid peroxide and conjugated diene compared to *Cyamopsis tetragonolobus* treated animals. Both the plants were thus found to be effective in reducing the oxidative stress.

**Table 1 - Levels of lipid peroxides and conjugated dienes in serum of experimental animals**

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (nmol/mg protein)</th>
<th>Conjugated Dienes (nmol/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2.80 ± 0.01</td>
<td>3.01 ± 0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>4.32 ± 0.01&lt;sup&gt;a*&lt;/sup&gt;</td>
<td>10.90 ± 0.13&lt;sup&gt;a*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>3.20 ± 0.01&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>7.80 ± 0.09&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.92 ± 0.02&lt;sup&gt;c*&lt;/sup&gt;</td>
<td>4.10 ± 0.06&lt;sup&gt;c*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group.
Statistical Significance: *p<0.001
Comparison: a – as compared with Group I; b – as compared with Group II;
 c – as compared with Group II

3. Enzymatic antioxidants in Serum
Table 2 shows the activities of superoxide dismutase, catalase and glutathione peroxidase in the serum of experimental animals. Group II animals showed significantly decreased (p<0.001) activities of enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase when compared to Group I animals. In experimental rats treated with *Cyamopsis tetragonolobus* (Group III) and *Cyperus rotundus* (Group IV), the activities of superoxide dismutase, catalase and glutathione peroxidase in serum significantly increased (p<0.001) reflecting the antioxidant potential of both the plant extracts. *Cyperus rotundus* treated (Group IV) animals showed near normal activities of these enzymatic antioxidants. The plants were thus found effective in preventing the oxidative stress formed by free radicals.

Table 2 - Activities of enzymatic antioxidants in serum of experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>CAT (µmol of H₂O₂ consumed/min/mg protein)</th>
<th>GPx (µmol of NADPH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>5.7 ± 0.01</td>
<td>5.57 ± 0.02</td>
<td>3.98 ± 0.02</td>
</tr>
<tr>
<td>Group II</td>
<td>3.1 ± 0.01</td>
<td>4.42 ± 0.02</td>
<td>1.50 ± 0.01</td>
</tr>
<tr>
<td>Group III</td>
<td>4.6 ± 0.20</td>
<td>5.43 ± 0.02</td>
<td>2.51 ± 0.01</td>
</tr>
<tr>
<td>Group IV</td>
<td>4.9 ± 0.13</td>
<td>5.47 ± 0.02</td>
<td>3.31 ± 0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group

Statistical Significance: *p<0.001
Comparison: a – as compared with Group I; b – as compared with Group II;
 c – as compared with Group II

4. Non-Enzymatic Antioxidants in Serum
Table 3 shows the levels of reduced glutathione, vitamin C and vitamin E in the serum of experimental animals. Group II animals showed significantly decreased (p<0.001) levels of non-enzymatic antioxidants such as reduced glutathione, vitamin C and vitamin E as compared to Group I animals. *Cyamopsis tetragonolobus* treated (Group III) and *Cyperus rotundus* treated (Group IV) animals showed significantly increased (p<0.001) levels of reduced glutathione, vitamin C and vitamin E. The plant extracts were found to be effective...
in reducing the oxidative stress by increasing the non-enzymatic antioxidant levels in experimental animals.

**Table 3 - Levels of non-enzymatic antioxidants in serum of experimental animals**

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µg/mg protein)</th>
<th>Vitamin C (mg/dl)</th>
<th>Vitamin E (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.81 ± 0.01</td>
<td>2.49 ± 0.01</td>
<td>1.74 ± 0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>2.12 ± 0.01 a</td>
<td>2.06 ± 0.01 a</td>
<td>0.80 ± 0.01 a</td>
</tr>
<tr>
<td>Group III</td>
<td>3.10 ± 0.01 b</td>
<td>2.38 ± 0.02 b</td>
<td>1.51 ± 0.01 b</td>
</tr>
<tr>
<td>Group IV</td>
<td>3.71 ± 0.02 c</td>
<td>2.36 ± 0.01 c</td>
<td>1.62 ± 0.01 c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 6 animals in each group

Statistical Significance: *p<0.001

Comparison: a – as compared with Group I; b – as compared with Group II;
            c – as compared with Group II

**DISCUSSION**

In our earlier studies, we have shown that the ethanolic extracts of *Cyperus rotundus* rhizomes and *Cyamopsis tetragonolobus* pods were found to be rich in various phytochemicals\(^{[23]}\) and showed good *in vitro* antioxidant potential\(^{[24]}\). In our previous *in vivo* study\(^{[13]}\), we have found that both *Cyperus rotundus* and *Cyamopsis tetragonolobus* prevented the incidence of diabetes-related changes in biochemical parameters including blood glucose, serum insulin, protein profile, lipid profile and enzymatic markers, thus, indicating their protective role against diabetic cataract. So, we planned to analyze the protective nature of both the plant extracts against oxidative stress *in vivo*.

The generation of reactive oxygen species play major role in etiology of diabetic complications\(^{[25]}\). Lipid peroxidation is a characteristic feature of chronic diabetes. The byproducts of lipid peroxidation are found to be increased in cell membranes of diabetic animals\(^{[26]}\). Oxidative stress produced by alloxan-induced diabetes was found to be significantly lowered due to the administration of *Aegle marmelos* that was evident from the significant decrease in lipid peroxide and conjugated diene levels\(^{[27]}\). Similarly, increase in serum lipid peroxidation due to diabetes was reduced by administration of ethanolic extract of *Wattakaka volubilis* leaf\(^{[28]}\). Administration of ethanolic extract of bark of *Tamarindus indica* (Fabaceae) causes significant decrease in lipid peroxidation products along with increased GSH in diabetic rats implicating its antioxidant property\(^{[29]}\). Thus, it should be noted from our study that oxidative stress condition has been increased by STZ in rats, but,
treatment with *Cyamopsis tetragonolobus* and *Cyperus rotundus* extracts prevented the experimental animals from oxidative stress.

There are many antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase that acts as main scavengers of free radicals produced due to several disease conditions\[^{30}\]. Many medicinal plants were shown to exhibit protective property via their antioxidant potential. Treatment with *Cyperus rotundus* and *Cyamopsis tetragonolobus* extracts showed significant restoration of SOD, CAT and GPx activity in lenses of *Lutjanus campechanus* under *in vitro* conditions that prevented the cataract development which may be due to the virtue of their antioxidant properties\[^{24}\]. The ethanolic root extract of *Kyllinga monocephala* (Cyperaceae) showed antioxidant activity against alloxan-induced diabetic rats by improving SOD and CAT level\[^{31}\]. The simultaneous incubation of *Abrus precatorius* (Fabaceae) extracts prevented the peroxidative damage caused by calcium, which is improved in goat lens as SOD, CAT and GPx levels increased\[^{32}\]. The decrease in enzymatic activities of catalase, superoxide dismutase and glutathione peroxidase as well as levels of non-enzymatic antioxidants such as vitamin C, vitamin E and reduced glutathione in diabetic rats are raised by administration of flower powder of *Cassia auriculata*, thus indicating its anti-oxidative effect\[^{33}\]. Similar to other medicinal plants, *Cyamopsis tetragonolobus* and *Cyperus rotundus* can also be considered for use as efficient antioxidant owing to its radical scavenging potential. Both the plants are found to be effective in reducing the oxidative stress created by diabetes-induced cataract.

Glutathione, vitamin C and vitamin E are valuable non-enzymatic antioxidants that play an important role in regulating the oxidative stress\[^{34}\]. Diabetes induced decrease in antioxidant activity such as GPx, SOD and GSH was attenuated by garlic (*Allium sativum*) administration in a dose-dependent manner and also delayed the progression of cataract\[^{35}\]. STZ administration created oxidative stress, thus affecting the antioxidant status in rats as seen by increase in GSH, ascorbic acid, SOD and catalase. Treatment with different doses of *Matricaria chamomilla* ethanolic extract significantly reduced the postprandial hyperglycemia and oxidative stress\[^{36}\]. Diabetic rats showed reduction in serum alphatocopherol levels\[^{37}\]. Thus, in our study, the animals with diabetes-induced cataract showed reduced levels of GSH, vitamin E and vitamin C. Treatment with *Cyamopsis tetragonolobus* (Group III) and *Cyperus rotundus* extracts (Group IV) reversed the reduction in non-
enzymatic antioxidant levels resembling several medicinal plants indicating the antioxidative potential of *Cyamopsis tetragonolobus* and *Cyperus rotundus*.

**CONCLUSION**

The increased levels of antioxidants in *Cyamopsis tetragonolobus* and *Cyperus rotundus* co-treated rats suggested that the free radical damage induced by STZ were normalized by both the plant extracts displaying their defensive role by inhibiting free radical production. It should be noted that treatment with ethanolic extract of *Cyamopsis tetragonolobus* and *Cyperus rotundus* helped in maintaining the cell redox status thereby increasing the efficacy of antioxidant defence system.

**REFERENCES**


