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EFFECT OF ETHANOLIC EXTRACTS OF *CYAMOPSIS TETRAGONOLOBUS* AND *CYPERUS ROTUNDUS* ON BIOCHEMICAL PARAMETERS OF DIABETIC CATARACT INDUCED WISTAR ALBINO RATS

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ABSTRACT

The present study evaluated the effect of ethanolic extracts of *Cyamopsis tetragonolobus* rhizomes and *Cyperus rotundus* pods on the biochemical parameters of diabetic cataract induced Wistar albino rats. Healthy rats were grouped into 4: Group I – Normal, Group II – Diabetic cataract induced animals (STZ-induced diabetic rats which developed cataract), Group III – *Cyamopsis tetragonolobus* extract co-treated animals and Group IV – *Cyperus rotundus* extract co-treated animals (Treatment for 19 weeks after STZ induction). Body weight and organ weight of cataract animals decreased while plant treatment prevented this loss. Increase in values of glucose, glycated haemoglobin, insulin, creatinine, urea and uric acid levels were observed in diabetic cataract induced rats, while plant co-treated groups restored these levels. The changes in lipid and protein profile in Group II rats were prevented by the plant treatment. SGOT, SGPT, ALP and LDH increased in Group II but plant treatment retained enzymatic activities. The results signify the plant's protective nature.

KEYWORDS: Diabetic cataract, *Cyamopsis tetragonolobus*, *Cyperus rotundus*, Streptozotocin, Biochemical parameter

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INTRODUCTION

Diabetic cataract is one of the major complications of diabetes which affects most of the patients. Cataractogenesis is one of the early secondary obstacle of diabetes mellitus which is a severe metabolic disorder characterized by hyperglycemia. Cataracts which occur in diabetic patients may cause blindness[1]. Though cataract surgery provides the possibility of regaining vision, it has some disadvantages. It will not resolve all the vision problems[2]. Medicinal plants are proven to be the resources of new drugs. They constitute a rich source of bioactive compounds and are found free from adverse effects but have exceptional pharmacological actions[3]. *Cyamopsis tetragonolobus*, commonly referred as cluster bean or guar bean is the source of guar gum. The guar bears pods which contain many seeds[4]. *Cyamopsis tetragonolobus* is traditionally used to cure inflammation, sprains and arthritis. They are also used as an antioxidant, antibilious and laxative agent[5]. *Cyperus rotundus* also known as nutgrass or nut sedge is a major weed. It has tuberous roots or rhizomes[6]. It is used for treating a wide range of diseases such as scabies, eczema, itching, fever, cough and asthma[7]. Many natural products and herbal medicines have been well recommended for the treatment of diabetes. Most of the plant extracts exhibited hypoglycaemic, hypolipidemic and antioxidant effects in experimental animals as well as in humans, which are helpful in treating diabetes and its associated complications[3]. In our present study, we have investigated the effect of the ethanolic extracts of *Cyamopsis tetragonolobus* pod and *Cyperus rotundus* rhizome on the biochemical parameters of diabetic cataract induced Wistar albino rats.

MATERIALS AND METHODS

(i) Plant Collection

The rhizomes of *Cyamopsis tetragonolobus* and pod (fruit) of *Cyperus rotundus* 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 a Botanist Prof. P. Jayaraman, Plant Anatomy Research Center, West Tambaram, Chennai, Tamil Nadu, India. The rhizome and pods were shade dried at room temperature for a week and made into coarse powder.

(iii) Extraction of Plants

20gm coarse powder of rhizomes of *Cyperus rotundus* and 20gm coarse powder of pods of *Cyamopsis tetragonolobus* were homogenized in 100ml of 90% ethanol separately using Waring blender. The extraction procedure was carried out as mentioned in our earlier study[8]. 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 given below:

Group I – Normal control (6 animals)

Group II – Diabetic cataract induced rats (6 animals)

Induction of Diabetic Cataract: Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (purchased from Sigma, USA) (60mg/kg body weight) dissolved in citrate buffer pH 4.5[9] and maintained for 20 weeks to develop cataract and the presence of cataract was confirmed using slit lamp biomicroscope.

Group III – After STZ induction for one week, co-treatment with *Cyamopsis tetragonolobus* pod extract was done for 19 weeks [200mg/kg body weight of the plant extract were orally fed to experimental animals] (6 animals)

Group IV - After STZ induction for one week, co-treatment with *Cyperus rotundus* rhizome extract was done for 19 weeks [200mg/kg body weight of

the plant extract were orally fed to experimental animals] (6 animals)

(vi) Collection of Blood and Organs

All the animals were killed by cervical decapitation after the experimental period. The blood was collected without EDTA for the separation of serum. The lens was removed from the eyes and homogenized with motor driven Teflon coated homogenizer in ice-cold 0.1M Tris-HCl buffer pH 7.4 to obtain 10% homogenate.

(vii) Body weight and Organ weight

The body weight of rats was checked using a top loader weighing balance. The body weight is expressed in grams. The vital organs such as eyes, liver, kidney, spleen, brain, pancreas and heart were collected. Blood was removed from the organs with the filter paper and their weights were assessed with an electric weighing balance. The organ weights were expressed in grams or milligrams.

(viii) Assessment of biochemical parameters in blood and serum

Blood glucose[10], serum glycated hemoglobin[11], total protein[12] and albumin[13] were estimated. Globulin present in serum can be calculated by subtracting the measured albumin from total protein. Serum insulin levels were assayed using a standard Mercodia Rat Insulin ELISA enzyme immunoassay kit from Mercodia, Sweden (cat.no-10-1124-01). The assay was performed according to manufacturer's protocol. Blood urea[14], serum uric acid[15] and creatinine[16] were also estimated. Lipid was extracted[17] from serum and cholesterol[18], triglyceride[19], free fatty acid[20,21], lipoprotein[22], and HDL[23] were estimated. LDL and VLDL levels were calculated by Friedewald formula. Activities of SGOT[24], SGPT[24], ALP[25] and LDH[26] in serum were also determined.

(ix) 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. Within group comparisons were performed with Tukey's ANOVA test. Significant differences between the control and experimental groups were assessed by the Student's t-test using Graph pad prism 6.0.

RESULTS

1. Cataract Incidence

The occurrence of cataract was observed after 20 weeks of STZ administration and confirmed by slit-lamp biomicroscope to investigate lenticular opacity. Diabetes-induced cataract gradually progressed in STZ-administered rats alone and the cataract was graded as cortical cataract (Figure 1).

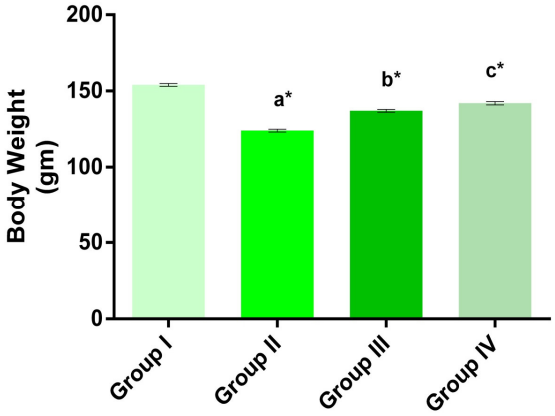
Figure 1
Diabetic Cataract Rat with clouding of eyes

The experimental animals co-treated with the ethanolic extract of *Cyamopsis tetragonolobus* pod and *Cyperus rotundus* rhizome for 19 weeks after STZ administration (after 1 week period) did not show the development of cataract.

2. Body Weight

Different groups of rats showing their body weight is depicted in Figure 2. Diabetes-induced cataract animals showed significant decrease ($p<0.001$) in body weight when compared with control animals. The ethanolic extract of *Cyamopsis tetragonolobus* treated animals and *Cyperus rotundus* treated animals were found to be have significant increase ($p<0.001$) in body weight when compared with diabetes-induced cataract animals.

Figure 2
Body weights of experimental animals



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
Group I - Control Animals
Group II - STZ-induced animals with Diabetic Cataract
Group III - Ethanolic extract of Cyamopsis tetragonolobus treated animals
Group IV - Ethanolic extract of Cyperus rotundus treated animals

Though, both plants were found to be effective in maintaining the body weight of STZ-induced animals, *Cyperus rotundus* treated animals showed slightly better results when compared with *Cyamopsis tetragonolobus* treated animals. Extract does not exert any side effects or toxic symptoms inferring its non-toxic nature. No death was observed.

3. Organ Weight

Different groups of rats with their organ weight is shown in Table 1.

Table 1
Organ weights of experimental animals

Groups	Eye Weight (gm)	Liver Weight (gm)	Kidney Weight (mg)	Pancreas Weight (mg)	Spleen Weight (mg)	Heart Weight (mg)	Lens Weight (mg)	Brain Weight (mg)
Group I	1.28±0.01	5.46±0.02	629±0.89	275±0.63	236±0.89	339±0.89	729±1.55	761±1.10
Group II	1.09±0.01[a*]	5.24±0.01[a*]	581±1.1[a*]	207±1.79[a*]	214±1.26[a*]	348±1.26[a*]	710±0.63[a*]	734±0.89[a*]
Group III	1.19±0.01[b*]	5.43±0.02[b*]	602±1.26[b*]	246±0.89[b*]	250±0.89[b*]	338±1.41[b*]	713±1.26[b*]	740±1.41[b*]
Group IV	1.18±0.01[c*]	5.44±0.02[c*]	610±1.79[c*]	270±0.89[c*]	225±1.10[c*]	340±1.26[c*]	723±1.41[c*]	748±1.41[c*]

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

There was a significant ($p<0.001$) decrease in weights of vital organs such as eye, liver, kidney, pancreas, spleen, heart, lens and brain of diabetes-induced cataract animals when compared with control animals. The ethanolic extract of *Cyamopsis tetragonolobus* treated animals and *Cyperus rotundus* treated animals were found to have significant increase ($p<0.001$) in the organ weights when compared with that of animals with diabetes-induced cataract.

4. Biochemical Parameters in Serum

4 (i). Biochemical profile in serum

Table 2 represents the changes of blood glucose, serum glycated haemoglobin, insulin, urea, uric acid and creatinine levels in samples of control and experimental animals. The levels of blood glucose, serum glycated haemoglobin, urea and uric acid were significantly increased ($p<0.001$) in Group II animals when compared with Group I animals. In plant treated animals, the levels of glucose, glycated haemoglobin, urea and uric acid were significantly less ($p<0.001$) when compared to Group II animals. *Cyperus rotundus* treatment decreased the blood glucose levels of animals to a greater extent than when compared to *Cyamopsis tetragonolobus* treatment even after STZ injection. The levels of glycated haemoglobin, urea and uric acid were significantly less ($p<0.001$) in Group III animals and Group IV animals. The levels of serum insulin and creatinine significantly decreased ($p<0.001$) in Group II animals when compared with Group I animals. The levels of serum insulin and creatinine in both plant treated animals increased significantly ($p<0.001$). *Cyperus rotundus* treatment was better in increasing the serum insulin levels when compared to that of *Cyamopsis tetragonolobus* treatment. It was noticed that the levels of glycated haemoglobin, creatinine, urea and uric acid were higher in *Cyamopsis tetragonolobus* treated plants than with *Cyperus rotundus* treatment.

Table 2

Levels of glucose, glycated hemoglobin, insulin, urea, uric acid and creatinine of experimental animals

Groups	Glucose (mg/dl)	Glycated Hemoglobin (%)	Insulin (µU/ml)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Group I	82.57±0.10	4.5±0.22	16.44±0.03	24.35±0.04	1.13±0.01	0.96±0.01
Group II	476.29±0.04 [a*]	8.4±0.21 [a*]	7.72±0.04 [a*]	38.61±0.04 [a*]	2.51±0.01 [a*]	0.34±0.01 [a*]
Group III	257.80±0.15 [b*]	5.0±0.63 [b*]	12.27±0.04 [b*]	25.48±0.08 [b*]	1.52±0.01 [b*]	1.10±0.02 [b*]
Group IV	132.11±0.01 [c*]	4.1±0.06 [c*]	13.75±0.05 [c*]	25.20±0.05 [c*]	1.47±0.02 [c*]	1.00±0.13 [c*]

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 (ii). Protein profile in serum

Table 3 represents the changes of total protein, albumin and globulin levels in serum samples of control and experimental animals.

Table 3

Levels of total protein, albumin and globulin in serum of experimental animals

Groups	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Group I	6.57 ± 0.04	4.25 ± 0.03	3.39 ± 0.05
Group II	5.44 ± 0.07 [a*]	2.64 ± 0.09 [a*]	2.29 ± 0.08 [a*]
Group III	5.70 ± 0.04 [b*]	3.30 ± 0.07 [b*]	3.38 ± 0.03 [b*]
Group IV	6.55 ± 0.04 [c*]	3.39 ± 0.09 [c*]	3.89 ± 0.06 [c*]

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

The levels of total protein, albumin and globulin were significantly decreased ($p<0.001$) in Group II animals when compared with Group I animals. In both plant treated animals, the total protein, albumin and globulin levels increased significantly ($p<0.001$) and reached near normal when compared to that of Group II animals. The levels of total protein, albumin and globulin were significantly more in Group IV animals when compared with that of Group III animals. Thus, *Cyperus rotundus* treatment was better in restoring the total protein, albumin and globulin levels.

4 (iii). Enzymatic markers in serum

Table 4 represents the activities of marker enzymes such as SGOT, SGPT, ALP and LDH in serum samples of control and experimental animals.

Table 4

Activities of enzymatic markers such as SGOT, SGPT, ALP and LDH in serum of experimental animals

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	LDH (IU/L)
Group I	84.1 ± 0.06	48.30 ± 0.11	153.25 ± 0.14	169.1 ± 0.11
Group II	110.2 ± 0.35 [a*]	67.69 ± 0.19 [a*]	298.77 ± 0.09 [a*]	278.0 ± 1.67 [a*]
Group III	93.0 ± 1.90 [b*]	34.10 ± 0.06 [b*]	232.02 ± 0.51 [b*]	185.0 ± 1.10 [b*]
Group IV	87.0 ± 2.28 [c*]	43.00 ± 1.41 [c*]	245.37 ± 0.02 [c*]	173.0 ± 2.10 [c*]

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

The activities of SGOT, SGPT, ALP and LDH increased significantly ($p<0.001$) in Group II animals when compared to that of Group I animals. The activities of these marker enzymes decreased significantly ($p<0.001$) in both plant treated animals as compared to Group II animals.

4 (iv). Lipid profile in serum

Table 5 represents the changes in lipid profile in serum samples of control and experimental animals.

Table 5

Changes in lipid profile in serum of experimental animals

Groups	Free fatty acid (mg/dl)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)
Group I	84.71 ± 0.01	69.1 ± 0.06	53.58 ± 0.05	29.88 ± 0.05	40.5 ± 0.01	16.41 ± 0.01
Group II	193.63 ± 0.02 [a*]	125.2 ± 0.14 [a*]	94.49 ± 0.03 [a*]	85.10 ± 0.09 [a*]	30.2 ± 0.06 [a*]	26.64 ± 0.02 [a*]
Group III	107.19 ± 0.01 [b*]	105.0 ± 1.67 [b*]	67.24 ± 0.03 [b*]	40.00 ± 2.83 [b*]	33.1 ± 0.09 [b*]	21.79 ± 0.03 [b*]
Group IV	97.07 ± 0.03 [c*]	90.0 ± 2.68 [c*]	58.30 ± 0.04 [c*]	35.00 ± 2.00 [c*]	38.4 ± 0.20 [c*]	20.29 ± 0.02 [c*]

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

The levels of free fatty acids, total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol significantly increased ($p<0.001$) in Group II animals, while HDL-cholesterol level decreased significantly ($p<0.001$) when compared to Group I animals. The levels of free fatty acids, total cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol lowered significantly ($p<0.001$) in Group III and Group IV animals when compared to that of Group II animals. The levels of HDL-cholesterol rose significantly ($p<0.001$) in both plant treated animals compared to Group II animals. From the results, it should be noticed that *Cyperus rotundus* treatment was fairly better in altering the lipid profile of experimental rats when compared with that of *Cyamopsis tetragonolobus* treatment.

DISCUSSION

Oral administration of ethanol extracts of *Cyperus rotundus* rhizomes did not lead to any changes in general behavior, mortality, weight gain, hematological and clinical blood chemistry parameters. Hence, these extracts did not cause oral acute (single dose of 5000 mg/kg) and sub-acute toxicity (repeated oral dose of 1000 mg/kg) in rats[27]. Thus, oral administration of ethanol extracts of *Cyperus rotundus* rhizomes can be considered safer. Hydro-alcoholic extract of *Cyamopsis tetragonolobus* possess hepato-protective activity against CCl_4-induced hepatotoxicity in rats and suggested that the effect is preventive in nature at oral dose of 250 mg/kg and 500 mg/kg[28]. Oral administration (dose of 500mg/kg body weight) of an ethanolic extract of *Cyamopsis tetragonoloba* (Guar) produced significant anti-ulcer, anti-secretory and cytoprotective effect in rats[29]. It is well-known that diabetes is very closely associated with weight loss. The body weights of diabetic cataract rats are lowered[30]. The same has been confirmed in a rat model during evaluation of early diabetes-induced biochemical changes in retina[31]. The body weight of STZ-induced diabetic rats improved on treatment with varied medicinal plants similar to our results. The oral feeding of water extract of fresh leaves of *Azadirachta indica* improved body weight of STZ-induced diabetic rats and reversed diabetic retinopathy[32]. Similarly, from our study also, it is observed that the treatment with both the plants prevented the loss of body weight induced by STZ. The relative weights of kidney and liver increased in STZ-induced diabetic albino rats, while the weight of pancreas remained unaffected[33]. The weight of the pancreas and kidney increased in STZ-diabetic rats, but treatment with fermented red ginseng seemed to decrease the overall weights with no significant difference in spleen weight of diseased and treated rats[34]. The relative organ weights of brain, liver, lungs, heart, kidney and spleen of *Lepidium sativum* seed powder treated Wistar rats showed comparable results with that of the normal rats[35]. The lens of diabetic cataract rats decreased, while treatment with *Trigonella* (Fabaceae) and vanadate resumed the lens weight to that of normal rats[30]. Our study also indicates the beneficial aspects of administering *Cyamopsis tetragonolobus* and *Cyperus rotundus* to prevent loss of body weight or organ weight that occur due to diabetic complications.

The aqueous extract of leaves and flowers of *Clitoria ternatea* (Fabaceae) exerted a hypoglycaemic effect in alloxan-induced diabetic rats as evidenced by reduction in serum glucose, glycated haemoglobin along with increased serum insulin[36]. Oral administration of *Annona squamosa* aqueous extract to diabetic rats significantly reduced blood glucose, urea, uric acid and creatinine[37]. The ethanol extract of *Chromolaena odorata* leaves reduced glucose, lipid profile and improved glucose and insulin tolerance as well as serum insulin and HDL-cholesterol levels when administered to STZ-induced diabetes and cataract in rats[38]. The aqueous root extract of *Pseudarthria viscida* (Fabaceae) restored the levels of protein and albumin in STZ-diabetic rats[39]. The levels of protein reduced in diabetic rats while treatment with *Annona squamosa* increased the protein levels and reached near normal. The levels of albumin and albumin/globulin ratio also decreased in diabetic animals while treatment restored these levels significantly[37]. Thus, in our study the reduction in protein profiles induced by STZ is restored by treatment with both the plants. The activity of ALP and aminotransferases ALT and AST lowered in STZ-induced diabetic rats while treatment of *Aspalathus linearis* (rooibos tea) restored their activities[40]. The increase in serum lactate dehydrogenase activity in streptozotocin-induced diabetic rats is reversed by aqueous extract of *Embelia ribes* Burm[41]. The ethanolic extracts of *Rheum emodi* rhizome treated for 30 days restored the activities of marker enzymes such as ALT, AST, ALP and LDH in serum to near control levels which rose due to induction of diabetes by alloxan in rats[42]. Hence, it can be noticed that the enzymatic marker levels were increased by STZ in diabetes-induced groups. But the plant treated groups

retained their enzyme activities despite induction of diabetes during the experimental period. Treatment with *Tinospora cordifolia* extract decreased serum total cholesterol, LDL-cholesterol and triglyceride significantly, while increase in serum HDL-cholesterol levels was observed. The ethanolic leaf extract of *Mucuna pruriens* (Fabaceae) improved the lipid profile of alloxan-induced diabetic Wistar rats[43]. Dietary supplementation with *Cyperus esculentus* (Cyperaceae) significantly improved the altered biochemical parameters such as changes in serum total lipids, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol levels in STZ-diabetic rats after four weeks of treatment[44]. The elevated levels of total cholesterol, triglyceride and LDL-cholesterol and a decrease in HDL-cholesterol levels in STZ-induced diabetic rats are modified as better lipid profile by administering aqueous extract perfusion of *Rosmarinus officinalis*[45]. Similarly, ethanol extract of *Wattakaka volubilis* possess significant antihyperlipidemic effect in alloxan-induced diabetic rats[46].

CONCLUSION

From the above results, it could be inferred that the biochemical changes signify that the ethanolic extract of *Cyamopsis tetragonolobus* and *Cyperus rotundus* has definite anti-cataract effect against STZ-induced diabetic cataract by delaying the cataractogenesis.

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CONFLICT OF INTEREST

Conflict of interest declared none

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