ORIGINAL CONTRIBUTION

Antiperoxidative and Antioxidant Effects of *Casearia Esculenta* Root Extract in Streptozotocin-Induced Diabetic Rats

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Oxidative stress is currently suggested to play as a pathogenesis in the development of diabetes mellitus. The present study was designed to evaluate the effect of *Casearia esculenta* root extract on oxidative stress-related parameters in streptozotocin (STZ) -induced diabetic rats. Antidiabetic treatment with *C. esculenta* root extract (45 days) significantly ($p < .05$) decreased thiobarbituric acid reactive substances (TBARS) and remarkably improved tissue antioxidants status such as glutathione (GSH), ascorbic acid (vitamin C) and α-tocopherol (vitamin E) in liver and kidney of STZ-diabetic rats. In diabetics rats, the activities of enzymatic antioxidants such as superoxide dismutase (SOD, EC 1.11.1.1) catalase (CAT, EC 1.11.1.6) were decreased significantly while the activity of glutathione peroxidase (GPx, EC 1.11.1.9) decreased in the liver and increased in the kidney. The treatment of diabetic rats with *C. esculenta* root extract over a 45-day period returned these levels close to normal. These results suggest that *C. esculenta* root extracts exhibit antiperoxidative as well as antioxidant effects in STZ-induced diabetic rats.

INTRODUCTION

Ancient Indian physicians termed diabetes mellitus as "Madumeha" (honey urine), and it has been treated orally with several medicinal plants or their extracts based on folk medicine [1]. The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycemic agents have stimulated greater interest in recent years [2]. Today, more than 200 traditional medici-

nal plants have been used for the treatment of diabetes mellitus and widely practiced in South India. Plant drugs are frequently considered to be less toxic and more free from side-effects than synthetic ones [3]. Synthetic oral hypoglycemic agents can produce a series of side-effects including hematological, gastro-intestinal reactions, hypoglycemic coma, and disturbances in liver and kidney metabolisms. In addition, these preparations are not ideal for use during pregnancy [4].

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†Abbreviations: CAT, catalase; DMRT, Duncan's multiple range test; GPx, glutathione peroxidase; GSH, glutathione; OFRs, oxygen free radicals; SOD, superoxide dismutase; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances..

Many herbs have been shown to have hypoglycemic action in both human and animals. *Casearia esculenta* Roxb. (Flacourtiaceae) is one such plant, popularly known as "kadala-zhinjill," "kottarkovai" in Tamil, "wild cowrie fruit" in English, and "saptarangi" in Sanskrit is a shrub richly distributed in the Konkan plateau in South India. In Indian traditional medicine, the plant has been a popular remedy for the treatment of diabetes mellitus [5-7], and our study drug is one of the major ingredients of D-400, the largest selling antidiabetic drug in India (Himalaya Drug Company, Bangalore) [8].

The first scientific study was undertaken by Gupta et al. [9], and they reported the hypoglycemic effect of this plant in rat and rabbits. Choudhury and Basu [10] then reported that *C. esculenta* root extract contained uncharacterized hypoglycemic factor(s) that reduced blood sugar level in experimental animals. Our laboratory preliminary research was highly encouraging and revealed that blood glucose level was significantly lowered after oral administration of *C. esculenta* root extract in normal animals, after a glucose load and in a streptozotocin (STZ)[†] -induced state. No harmful side effects were observed throughout the study [11], and the study also extended to explore the possibility of the antihyperglycemic property of *C. esculenta* root extract in a STZ-diabetic state [12], plasma antioxidant status in STZ-diabetic rats [13], erythrocyte redox status in STZ-diabetic rats [14], hypolipidaemic action in control and STZ-diabetic rats [15], and protein metabolism and marker enzymes in STZ-induced diabetic rats [16].

To our knowledge, no detailed investigations had been carried out to shed light on the antioxidant status in the liver and kidney of diabetic rats injected with STZ (50 mg/kg body weight) and after treatment with *C. esculenta* root extract for 45 days, and this will yield valuable information on whether the crude drug can improve the tissue antioxidant status or not, particularly in liver and kidney. Thus, the present investigation envisages studying the effect of *C. esculenta* root extract on liver and kidney antioxidant status in control and STZ-diabetic rats. The effects produced by *C. esculenta* were compared with the standard drug glibenclamide.

MATERIALS AND METHODS

Plant material

The root of *C. esculenta* (Syn = *Casearia ovata*) was collected from Western ghats of Tamil Nadu, and the plant was botanically authenticated by Dr. C. Chelladurai, research officer, Survey Medicinal Plant Unit (SMPU), Central Council for Research in Siddha and Ayurvedic, Siddha Medical College, Palayamkottai, Tamilnadu. Voucher specimen was deposited in the (AU2145) Department of Botany, Annamalai University, Annamalainagar, Tamilnadu. The plant root was air-dried at 25°C, and the dried root was made into a fine powder with an auto-mix blender. The powder was kept in deep freezer until the time of use.

Preparation of aqueous extract

One hundred grams of dry fine powder was suspended in 250 ml of water for two hours and then boiled at 60° to 65°C for 30 minutes (since boiled decoction of the root of this plant has been used as remedy for diabetes). The extract was preserved, and the processes were repeated three times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40°C yielded 12 percent semi-solid extract.

Drugs and chemicals

STZ was obtained from the Sigma Chemical Company (St. Louis, Missouri). All other chemicals used were of analytical grade.

Animals

Male Wistar albino rats (weighing 140 g to 160 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. The animals were maintained at Central Animal House and fed a standard diet (Hindustan Lever, Bangalore) and water *ad libitum*. All studies were conducted in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* [17], and the study was approved by the Ethical Committee of Rajah Muthaih Medical College and Hospital, Annamalai University, Annamalainagar.

Experimental induction of diabetes

Adult (9-week-old) male Wistar rats were made diabetic with an intraperitoneal injection of STZ (50 mg/kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5). STZ-injected animals exhibited massive glycosuria and hyperglycemia within a few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, 96 hours after injection with STZ. Albino rats with a blood glucose level above 240 mg/dl were considered to be diabetic and were used in the experiment. Six rats were injected with 2 percent gum acacia alone that served as control.

Animal allotment

After the induction of diabetes, the rats were divided into five groups of six animals each:

Group I: Control rats received vehicle solution (2 percent gum acacia).

Group II: Diabetic control.

Group III: Diabetic rats received *C. esculenta* root extract (200 mg/kg body weight) in 2 percent gum acacia using an intragastric tube daily for 45 days.

Group IV: Diabetic rats given *C. esculenta* root extract (300 mg/kg body weight) in 2 percent gum acacia using an intragastric tube daily for 45 days.

Group V: Diabetic rats received glibenclamide orally (600 µg/kg body weight) as aqueous solution using an intragastric tube daily for 45 days.

At the end of the experimental period, rats were fasted for 12 hours, then sacrificed by cervical decapitation. Fasting blood samples were collected from the sacrificed animals in tubes with heparin. Plasma samples were obtained by centrifugation and stored at -20ºC until measured. The liver and kidney were carefully removed, weighed, and washed using chilled, isotonic saline and blotted individually on ash-free filter paper. The liver and kidney were minced and homogenized in 0.1 M Tris-HCl buffer (pH 7.4) in a potter-Elvehjem type homogenizer. The homogenate was used for the estimations of reduced glutathione (GSH) [18], ascorbic acid (vitamin C) [19], α-tocopherol (vitamin E) [20], thiobarbituric acid reactive substances (TBARS) [21], protein [22], superoxide dismutase (SOD) [23], catalase (CAT) [24], and glutathione peroxidase (GPx) [25].

Statistical analysis was performed using the SPSS software package (Statistical Package for the Social Sciences, United States). Data are presented as means with their standard deviations, and the data were analyzed using analysis of variance (ANOVA). The group means were compared with Duncan's Multiple Range Test (DMRT).

RESULTS

Tables 1 and 2 show the tissue concentrations of GSH, ascorbic acid, α-tocopherol, and TBARS in the liver and kidney of control and STZ-diabetic rats. There was a significant elevation in blood glucose [11-16] and TBARS (liver and kidney) ($p < .05$) and significant reduction ($p < .05$) in non-enzymatic antioxidants in liver and kidney of STZ-diabetic rats when

Table 1. Effect of *C. esculenta* root extract on GSH, ascorbic acid, α-tocopherol, and TBARS in the liver of control and experimental animals.

Group	Treatment (dose/kg body weight)	GSH (µg/mg protein)	Vitamin C (µg/mg protein)	Vitamin E (µg/mg protein)	TBARS (nmoles/mg protein)
I	Control (2% gum acacia)	7.14 ± 1.01[a]	0.53 ± 0.12[a]	3.37 ± 0.26[a]	1.34 ± 0.07[a]
II	Diabetic control	4.62 ± 0.67[a]	0.33 ± 0.06[b]	1.35 ± 0.14[b]	3.42 ± 0.42[b]
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	5.21 ± 0.38[b]	0.37 ± 0.08[b,c]	2.33 ± 0.15[c]	2.63 ± 0.27[b]
VI	Diabetic + *C. esculenta* (300 mg/kg body wt.)	6.91 ± 0.42[a]	0.46 ± 0.11[a,b]	3.26 ± 0.30[a]	1.77 ± 0.22[a]
V	Diabetic + glibenclamide (600 mg/kg body wt.)	7.14 ± 1.03[a]	0.51 ± 0.12[a,c]	3.29 ± 0.31[a]	1.56 ± 0.22[a]

Values are mean ± SD for six animals in each group. Values not sharing common superscripts differ significantly at $p < .05$, Duncan's multiple range test.

Table 2. Effect of *C. esculenta* root extract on GSH, ascorbic acid, α-tocopherol, and TBARS in the kidney of control and experimental animals.

Group	Treatment (dose/kg body weight)	GSH (µg/mg protein)	Vitamin C (µg/mg protein)	Vitamin E (µg/mg protein)	TBARS (nmoles/mg protein)
I	Control (2% gum acacia)	7.14 ± 1.01[a]	0.53 ± 0.12[a]	3.37 ± 0.26[a]	1.34 ± 0.07[a]
II	Diabetic control	4.62 ± 0.67[a]	0.33 ± 0.06[b]	1.35 ± 0.14[b]	3.42 ± 0.42[b]
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	5.21 ± 0.38[b]	0.37 ± 0.08[b,c]	2.33 ± 0.15[c]	2.63 ± 0.27[b]
VI	Diabetic + *C. esculenta* (300 mg/kg body wt.)	6.91 ± 0.42[a]	0.46 ± 0.11[a,b]	3.26 ± 0.30[a]	1.77 ± 0.22[a]
V	Diabetic + glibenclamide (600 mg/kg body wt.)	7.14 ± 1.03[a]	0.51 ± 0.12[a,c]	3.29 ± 0.31[a]	1.56 ± 0.22[a]

Values are mean ± SD for six animals in each group. Values not sharing common superscripts differ significantly at $p < .05$, Duncan's multiple range test.

compared to controls. Administration of *C. esculenta* root extract (200 and 300 mg/kg body weight) and glibenclamide for a period of 45 days decreased the glucose levels significantly and improved the tissue antioxidant status significantly ($p < .05$). *C. esculenta* extract at a dose of 300 mg/kg body weight was more effective then the other dose 200 mg/kg body weight.

Tables 3 and 4 show the activity of enzymatic antioxidants such as SOD, CAT, and GPx in the liver and kidney of normal and STZ-diabetic animals. There was a significant reduction in the activity of SOD and CAT in the liver and kidney of diabetic rats while the activity of GPx decreased in the liver and increased in the kidney in diabetic rats. Administration of *C. esculenta* root extract (200 and 300 mg/kg body weight) and glibenclamide significantly brought the enzyme activity to near normal values.

Table 3. Effect of *C. esculenta* root extract on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the liver of control and experimental animals.

Group	Treatment (dose/kg body weight)	SOD (U^a/mg protein)	CAT (U^b/mg protein)	GPx (U^c/mg protein)
I	Control (2% gum acacia)	9.57 ± 0.48^a	$71.67 \pm 2.89^{b,d}$	5.56 ± 0.73^a
II	Diabetic control	5.75 ± 1.27^b	38.09 ± 2.96^a	3.17 ± 0.87^b
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	8.59 ± 1.12^a	56.59 ± 3.31^c	6.79 ± 1.50^c
VI	Diabetic + *C. esculenta* (300 mg/kg body wt.)	8.97 ± 0.77^a	68.65 ± 3.38^b	5.12 ± 0.48^a
V	Diabetic + glibenclamide (600 mg/kg body wt.)	9.13 ± 0.72^a	73.64 ± 4.49^d	5.27 ± 0.45^a

U^a = enzyme concentration required to inhibit the nitroblue tetrazolium 50 percent. U^b = μmol of H_2O_2 consumed/min. U^c = μg of GSH utilized/min. Values are mean ± SD for six animals each. Values not sharing common superscripts differ significantly at $p < .05$, Duncan's multiple range test.

Table 4. Effect of *C. esculenta* root extract on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the kidney of control and experimental animals.

Group	Treatment (dose/kg body weight)	SOD (U^a/mg protein)	CAT (U^b/mg protein)	GPx (U^c/mg protein)
I	Control (2% gum acacia)	12.96 ± 0.92^a	34.51 ± 2.29^a	3.56 ± 0.20^a
II	Diabetic control	7.77 ± 1.40^b	19.47 ± 2.20^b	5.96 ± 1.84^b
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	11.32 ± 1.45^c	27.71 ± 2.54^c	4.24 ± 0.35^a
VI	Diabetic + *C. esculenta* (300 mg/kg body wt.)	12.43 ± 1.11^{ac}	33.76 ± 2.45^a	3.48 ± 0.23^a
V	Diabetic + glibenclamide (600 mg/kg body wt.)	11.39 ± 0.74^c	33.47 ± 2.77^a	3.44 ± 0.20^a

U^a = enzyme concentration required to inhibit the nitroblue tetrazolium 50 percent. U^b = μmol of H_2O_2 consumed/min. U^c = μg of GSH utilized/min. Values are mean ± SD for six animals each. Values not sharing common superscripts differ significantly at $p < .05$, Duncan's multiple range test.

DISCUSSION

STZ is a nitrosurea compound produced by S*treptomyces achromogenes*, which specifically induces DNA strand breakage in ß-cells causing diabetes mellitus. Therefore, the STZ-diabetic model has been widely employed to induce diabetes in experimental animals [26]. It has been earlier reported that the oral administration of aqueous extract of *Salvia lavandulifolia* [27], *Gymnema sylvestre* [28, 29], and D-400 (herbo-mineral preparation) [30] has significantly increased the numbers and size of the pancreatic islets in STZ-induced diabetic animals. Based on the earlier studies [31-32], the dose of STZ has been chosen to induced diabetes mellitus in the present study.

Raised glucose level [10-15] can induce oxidative stress via the generation

of oxygen free radicals (OFRs). There are many ways by which hyperglycemia may increase the generation of OFRs, such as glycoxidation, polyol pathway, prostanoid biosynthesis, and protein glycation [34]. There is also ample evidence that elevation in glucose concentration may depress natural antioxidant defense such as ascorbic acid and GSH [34, 35]. The imbalance between the generation of OFRs and an antioxidant defense system may increase the oxidative stress and lead to the damage of macromolecules such as DNA, proteins, or lipids.

Lipid peroxidation is a free radical-mediated process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxide are found in plasma and tissues. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species, and decreased level of antioxidant defenses such as GSH and ascorbic acid [36].

In the present study, there is an increase in the level of TBARS and a decrease in the enzymatic and non-enzymatic antioxidants in the liver and kidney of STZ-diabetic rats. Increased levels of TBARS suggest increasing OFRs. Lipid peroxide-mediated tissue damage has been observed in the development of Type II and Type I diabetes. Sundaram et al. (1996 [37]) have reported that the concentration of lipid peroxides increases in the kidney of diabetic rats. An increased level of TBARS is an index of lipid peroxidation. Our study shows that administration of *C. esculenta* extract tends to bring the kidney and liver TBARS back to near normal. Increased lipid peroxidation under diabetic condition can be due to the increased oxidative stress in the cells as a result of depletion of antioxidants scavenger systems as reported by Anuradha and Selvem [38].

GSH is known to protect the cellular system against the toxic effects of lipid peroxidation [39]. The total glutathione concentrations decline in liver and kidney of diabetic rats by 39 percent and 35 percent, respectively, which represent increased utilization due to oxidative stress. *C. esculenta* and glibenclamide-treated animals show significant reversal of glutathione content in the liver and kidney.

Ascorbic acid is known to act as an antioxidant both in *in vivo* and *in vitro*. It functions as a free-radical scavenger and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid plays an important role in detoxification of reactive intermediates produced by cytochrome P_{450}, which detoxify xenobiotics. Reduction in tissue ascorbic acid was observed in STZ-diabetic rats.

The decrease could have been due to increased utilization of ascorbic acid as an antioxidant defense against increased reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of ascorbic acid [40].

α-Tocopherol, a lipid soluble, chain-breaking antioxidant was significantly decreased in liver and kidney of STZ-diabetic rats. *C. esculenta* and glibenclamide treatment tends to bring the α-tocopherol levels to near normal value. Higuchi (1982 [42]) observed a decreased hepatic α-tocopherol in rats with STZ-induced diabetes. These results suggest that the demand for the antioxidant vitamin E is increased due to the activation of free radical related metabolism in diabetes. Impaired generation of naturally-occurring antioxidants (GSH, ascorbic acid, and α-tocopherol) results in increased oxidative injury by failure of protective mechanisms. There is increased flux of glucose through the polyol pathway, which is hyperactive in hyperglycemia [42].

STZ-induced hyperglycemia induces generation of $O_2{}^-$ and hydroxyl radicals

($^{\bullet}$OH), which induces various injuries in surrounding organs and plays an important role in several clinical disorders. Any compound with rich antioxidant properties, might contribute toward the partial or total alleviation of organ damage. Therefore, removal of $O_2^{\bullet-}$ and $^{\bullet}$OH is probably one of the effective defenses of a living body against diseases. The elevated levels of SOD clearly shows that *C. esculenta* extracts contains free radicals-scavenging activity, which could exert a beneficial action against the pathological alteration caused by the presence of $O_2^{\bullet-}$ and $^{\bullet}$OH [43].

A significant decrease in SOD and CAT activities is observed in the liver and kidney of diabetic rats when compared with control animals. The decreased activity of catalase in the liver and kidney of STZ-diabetic rats could be due to the increased endogenous production of superoxide anions. H_2O_2 is toxic by itself and can be a precursor of other toxic species. It can react with $O_2^{\bullet-}$ to form $^{\bullet}$OH and result in increased lipid peroxidation and hence higher TBARS level [44]. H_2O_2 also yields $^{\bullet}$OH by metal (iron or copper) catalyzed Fenton/Haber-Weiss reaction. Increased Cu level has been observed in diabetic animals [45]. Cu can also catalyze Fenton reaction to generate $^{\bullet}$OH radical [46]. The $^{\bullet}$OH radical is also thought to be the primary reactive molecule in the redox activation of enzymes. Anuradha et al. [39] reported increased $^{\bullet}$OH radical production and increased hydroperoxide in kidney of allaxon-induced diabetic rats. Recently, Sulahudeen [47] reported that H_2O_2 induced lipid peroxidation causes renal epithelial cell injury.

Catalase causes reduction of H_2O_2 whereas GPx reduces H_2O_2 and lipid peroxides. The GPx in the the kidney of the the diabetic rat increased as compared to the control. The decreased catalase activity and increased GPx activity in the kidney suggests that there may be compensatory mechanism among the antioxidant enzymes in response to increased oxidative stress so

that tissues lacking significant catalase activity may be critically dependent on activity of GPx. Christophersen [48] reported that GPx has broader protective spectrum than catalase in catalyzing the reduction of both H_2O_2 and other hydroperoxides including lipid peroxides. Shull et al. [49] reported an increase in GPx mRNA at higher concentration of H_2O_2. It has been reported that pentose phosphate pathway is activated in diabetic kidney to meet the demand for ribose-5-phosphate utilized for nucleotide synthesis and for NADPH required for reductive biosynthesis [50]. An abundant supply of NADPH, a substrate for the glutathione reductase cycle may be related to the increased activity of GPx in diabetic kidney.

Considering our results, increased activities of SOD, CAT, and GPx in the liver and kidney of diabetic rats fully confirm that *C. esculenta* does extend a clear protective action against STZ-induced diabetic rats. This action is predominantly due to the extract and could involve a mechanism related to scavenging activity.

In the present study, we have shown that *C. esculenta*, an indigenous antidiabetic herb, shows potent antioxidant and antidiabetic properties, which could be due to the presence of potent antihyperglycaemic factor(s). Further study is underway in our laboratory to isolate the active principle and to study the mechanism of its action.

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