Ind J Clin Biochem (Oct-Dec 2010) 25(4):429–434
DOI 10.1007/s12291-010-0040-z

ORIGINAL ARTICLE

Anti-Oxidative Effect of *Cassia auriculata* on Streptozotocin Induced Diabetic Rats

N. Jeyashanthi · V. Ashok

Received: 3 February 2009 / Accepted: 9 October 2009 / Published online: 14 September 2010

Abstract The anti oxidative effect of administration of 100 mg/kg bw and 200 mg/kg bw of the flower powder of *Cassia auriculata* (CFP) for 45 days to normoglycemic and diabetic rats (streptozotocin induced) was studied. Anti oxidative effect was not observed in normoglycemic rats in the experiment. There was significant ($P > 0.05$) increase in the level of Thio Barbituric Acid Reactive Substances (TBARS), hydroperoxide and conjugated dienes and significant ($P > 0.05$) decrease in the catalase, superoxide dismutase and glutathione peroxidase activities and in the level of ascorbic acid, vitamin E and reduced glutathione in diabetic rats. The flower powder of *Cassia auriculata* significantly ($P > 0.05$) decreased the TBARS, hydroperoxide and conjugated dienes and increased the antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) and non enzymic anti oxidants (ascorbic acid, vitamin E and reduced glutathione). The antioxidatve effect of 200 mg/kg bw CFP was significantly ($P > 0.05$) better than 100 mg/kg bw CFP and the reference drugs (tolbutamide and metformin). The mode of action of CFP remains to be elicited.

N. Jeyashanthi (✉) · V. Ashok
Department of Biomedical Engineering, Velalar College of Engineering and Technology, Erode, Tamilnadu 638052, India
e-mail: njeyashanthi1316@yahoo.com

V. Ashok
e-mail: ashok_tronics@yahoo.com

Keywords Anti oxidative · *Cassia auriculata* · Streptozotocin induced diabetes · Enzymic antioxidants · Non enzymic anti oxidants

Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which the ability to oxidize and utilize carbohydrates is lost as a result of disturbances in normal insulin mechanism. Clinical and experimental evidences indicate that free radicals play important roles in many physiological and pathological conditions [1]. Oxidative stress is a disturbance in the balance between the production of the reactive oxygen species (free radicals) and antioxidant defenses, which may lead to tissue injury [2]. There is considerable evidence that oxidative stress is implicated in the development of diabetic complications [3]. The mechanisms behind the apparent increased oxidative stress in diabetes are not entirely clear. Accumulating evidence points to a number of inter related mechanisms increasing production of free radicals such as super oxide or increasing production of reactive oxygen and nitrogen species or decreasing anti oxidant status [4]. These mechanisms include glycoxidation and formation of advanced glycation products (AGE), activation of the polyol pathway and glutathione redox status ascorbate metabolism antioxidant enzyme inactivation and perturbation in the nitric oxide and prostaglandin metabolism [5]. The flower of *Cassia auriculata* is being used as popular remedy for the treatment of diabetes mellitus in Ayurveda and Siddha medicine [6] .

The present study was planned to evaluate the anti oxidative effect of anti diabetic herbal drug *Cassia auriculata* flower powder in streptozotocin induced diabetic rats.

Materials and methods

Preparation of *Cassia auriculata* Flower Powder (CFP)

Fresh flower of *Cassia auriculata* was collected from SKM Herbal Research Centre, Erode, Tamil Nadu and dried in shade. The flowers weighing 1 kg were finely powdered in a mill, sieved with a fine mesh and stored in an airtight container. The containers were kept in a deep freezer till the time of use.

Experimental design

Healthy male Wistar rats (bw 250–300 g) were obtained from Central Animal House, Raja Muthiah Medical College, Annamalai University, Chidambaram. The animals were housed in a clean and well ventilated animal house. They were fed with a standard pelleted diet (Gold Mohur, India) and drinking water ad libitum. The rats were divided into eight groups of ten rats each to determine the anti oxidative effect of flower powders of *Cassia auriculata*

Group I: Control animals receiving standard diet.
Group II: Animals receiving CFP(100 mg/kg bw) in 1 ml distilled water orally daily for 45 days.
Group III: Animals receiving CFP (200 mg/kg bw) in 1 ml distilled water orally daily for 45 days.
Group IV: Diabetic rats receiving standard diet.
Group V: Diabetic rats receiving CFP (100 mg/kg bw) in 1 ml distilled water orally daily for 45 days.
Group VI: Diabetic rats receiving CFP (200 mg/kg bw) in 1 ml distilled water orally daily for 45 days.
Group VII: Diabetic rats receiving bw Tolbutamide (200 mg/kg bw) in 1 ml distilled water orally daily for 45 days.
Group VIII: Diabetic rats receiving Metformin (10 mg/kg bw)in 1 ml distilled water orally daily for 45 days.

Induction of Diabetes Mellitus in Experimental Animals

Diabetes mellitus was induced in Group IV–VIII by a single intraperitoneal injection of streptozotocin (Upjohn company, Kalamazoo, MI, USA) dissolved in 0.1 M citrate buffer, pH 4.5 at a dose of 70 mg/kg body weight and after 15 days diabetes was confirmed by the presence of high blood and urine glucose level. Control rats were given a vehicle injection at the same time when the diabetic condition was induced in experimental animals.

Estimations

The animals were sacrificed by cervical dislocation on 46th day of the experiment and blood, liver and kidney were collected and stored at 4°C for different biochemical estimations. MDA (TBARS) [7], Hydroperoxides [8], conjugated dienes [9] were estimated in blood, liver and kidney. Superoxide dismutase activity was determined by the method of Kakkar et al. [10]. The activity of catalase was determined by the method of Sinha [11]. Glutathione peroxidase was estimated by the method of Rotruck et al. [12.]. The concentrations of antioxidants reduced glutathione [13] and ascorbic acid [14] were also estimated.

Statistical Analysis

All the data were statistically evaluated and the significance calculated using two ways 'ANOVA'. All the results were expressed as mean ± SD.

Results and Discussion

There is increasing demand by patients to use natural products with antidiabetic activity. This is because insulin cannot be used orally. Besides, certain oral hypoglycemic agents are not effective in lowering the blood sugar in chronic diabetic patients [15]. This is the main reason for the persistent interest all over the world to explore alternative remedies. *Cassia auriculata* is one of the herbs used in herbal formulations to treat diabetes [16]. *Cassia auriculata* alone also has antidiabetic and anti lipidemic effect [17]. The anti oxidative effect of this herb on diabetes was investigated on streptozotocin-induced diabetes mellitus in rats in the present study.

Changes in the Levels of TBARS, Hydroperoxides and Conjugated Dienes

The level of TBARS, hydroperoxides and conjugated dienes in serum, liver and kidney of control and experimental diabetic rats consequent to the administration of CFP is depicted in Table 1.

The levels of TBARS, hydroperoxides and conjugated dienes in plasma and liver and kidney were significantly ($P < 0.05$) higher in streptozotocin diabetic rats when compared to control rats. The rise of the lipid peroxidation observed in the diabetic rats agrees with the results of several other studies conducted on diabetic rats and human subjects [18]. Maxwell et al. [19] have reported that lipid peroxidation is one of the characteristic features of chronic diabetes. Byproducts of lipidperoxidation, such as conjugated dienes, hydroperoxides and TBARS are increased in cell membranes in diabetic animals. Santini et al. [20] reported that the diabetic patients had significantly increased levels of hydroperoxide, conjugated dienes and TBARS compared with controls.

Table 1 Effect of CFP on the level of TBARS, hydroperoxides and conjugated dienes

Groups	TBARS			Hydroperoxides			Conjugated dienes		
	Plasma (nmol/dl)	Liver (nmolg/g)	Kidney (nmol/g)	Plasma (nmol/dl)	Liver (nmol/g)	Kidney (nmol/g)	Plasma(nmolg/dl)	Liver (nmol/g)	Kidney (nmol/g)
I	175.3 ± 12.3[a]	130.3 ± 9.2[a]	149.2 ± 7.3[a]	1.83 ± 0.04[a]	2.45 ± 0.12[a]	4.36 ± 0.21[a]	3.01 ± 0.19[a]	7.25 ± 0.54[a]	3.86 ± 0.26[a]
II	180.8 ± 11.9[a]	134.6 ± 8.7[a]	155.7 ± 8.9[a]	1.79 ± 0.05[a]	2.39 ± 0.13[a]	4.48 ± 0.19[a]	3.25 ± 0.18[a]	7.30 ± 0.49[a]	3.72 ± 0.19[a]
III	176.2 ± 12.5a	129.7 ± 9.6[a]	143.2 ± 8.6[a]	1.80 ± .06[a]	2.40 ± 0.15[a]	4.29 ± 0.18[a]	3.15 ± 0.21[a]	7.29 ± 0.52[a]	3.83 ± 0.21[a]
IV	389.6 ± 19.8[b]	210.1 ± 10.3[b]	210.5 ± 10.8[b]	3.52 ± 0.07[b]	6.01 ± 0.13[b]	6.98 ± 0.22[b]	6.25 ± 0.23[b]	10.68 ± 0.49[b]	7.23 ± 0.29[b]
V	265.5 ± 14.6[c]	182.6 ± 9.8[c]	186.4 ± 8.3[c]	2.50 ± 0.05[c]	4.28 ± 0.14[c]	5.25 ± 0.21[c]	5.24 ± 0.17[c]	9.25 ± 0.43[c]	5.24 ± 0.21[c]
VI	182.4 ± 14.6[a]	131.2 ± 8.9[c]	151.9 ± 9.4[a]	1.91 ± 0.05[a]	2.54 ± 0.17[a]	4.39 ± 0.19[a]	3.31 ± 0.16[a]	7.38 ± 0.51[a]	3.75 ± 0.18[a]
VII	256.9 ± 13.2[c]	173.2 ± 7.6[c]	187.6 ± 9.7[c]	2.34 ± 0.04[c]	4.58 ± 0.16[c]	5.36 ± 0.17[c]	4.69 ± 0.15[c]	9.12 ± 0.48[c]	5.35 ± 0.17[c]
VIII	272.8 ± 10.8[c]	165.8 ± 8.7[c]	171.2 ± 8.6[c]	2.01 ± 0.06[c]	4.01 ± 0.14[c]	5.01 ± 0.15[c]	4.01 ± 0.14[c]	8.95 ± 0.39[c]	4.69 ± 0.19[c]

Values are mean ± SD for 10 rats. Values with different superscripts differ significantly ($P < 0.05$)

I control, *II* C + CFP (100 mg), *III* C + CFP (200 mg), *IV* diabetic (D), *V* D + CFP (100 mg), *VI* D + CFP (200 mg), *VII* D + Tolbutamide (100 mg), *VIII* D + Metformin (10 mg)

[a] Significantly ($P < 0.05$) different from diabetic rats

[b] Significantly ($P < 0.05$) different from normal rats

[c] Significantly ($P < 0.05$) different from normal and diabetic rats

In diabetic condition, the process of free radical production may be deleterious due to increased oxidative stress. An increase in lipid peroxides in plasma may be one of the important factors in the development of vascular complications and atherosclerosis in diabetes mellitus. High lipid peroxide concentration in serum is found in diabetic patients, who are a high cardiovascular risk group [21].

Lipid peroxide mediated tissue damage has been observed in both type 1 and type 2 diabetes. The reactive compounds of peroxidation cause peroxidation of lipids resulting in the formation of hydroperoxy fatty acids and endoperoxides. Hydroxyperoxide and an oxidant can form free radicals with ferrous ion and oxygen radicals. These free radicals can attack the phospholipids of membrane causing lipid peroxidation [22]. Increased concentration of lipid peroxide in the liver can result in decreased activity of cytochrome p_{450} and cytochrome b_5, and this may affect the drug metabolizing activity in chronic diabetes The increased concentration of lipid peroxidation byproducts (TBARS, conjugated dienes and hydroperoxide) in the kidney of diabetic rats may be due to increased breakdown of lipid constituents of renal membrane, thus altering the membrane integrity and function [23].

Oral administration of CFP 100 and 200 mg to streptozotocin-diabetic rats caused a significant ($P < 0.05$) decrease in the level of TBARS, conjugated dienes and hydroperoxides in plasma, liver and kidney as compared to that of diabetic rats. This may be due to the anti-peroxidative effect of the components present in CFP. The reference drugs tolbutamide and metformin also decreased the level of TBARS, conjugated dienes and hydroperoxides in plasma, liver and kidney of streptozotocin-induced diabetic rats significantly ($P < 0.05$). Both the concentrations of CFP showed better effect than tolbutamide and metformin in this respect. The CFP at the concentration of 200 mg is significantly more effective in lowering the level of TBARS in plasma and tissues than CFP at the concentration of 100 mg.

Changes in the Level of Enzymatic Antioxidants in Diabetic Experimental Rats

Table 2 represents the activities of antioxidant enzymes SOD, CAT and GPx. In control rats treated with CFP 100 mg and 200 mg/kg bw activities of the antioxidant enzymes were not significantly altered in plasma, liver and kidney.

The activities of SOD, CAT and GPx in plasma, liver and kidney of diabetic rats were significantly decreased when compared with those of control rats. An imbalance between the production and scavenging of free radicals can result in increased oxidative stress. Increased free radical

Table 2 Effect of CFP on the activity superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)

Groups	SOD U/mg protein/min			CAT U/mg protein/min			GPx U/mg protein/min		
	Plasma	Liver	Kidney	Plasma	Liver	Kidney	Plasma	Liver	Kidney
I	175.3 ± 8.2[a]	130.3 ± 6.4[a]	149.2 ± 5.2[a]	1.83 ± 0.02[a]	2.45 ± 0.05[a]	4.36 ± 0.19[a]	3.01 ± 0.12[a]	7.25 ± 0.19[a]	3.86 ± 0.19[a]
II	180.8 ± 8.1[a]	134.6 ± 5.9[a]	155.7 ± 4.9[a]	1.79 ± 0.01[a]	2.39 ± 0.04[a]	4.48 ± 0.21[a]	3.25 ± 0.14[a]	7.30 ± 0.23[a]	3.72 ± 0.17[a]
III	176.2 ± 7.9a	129.7 ± 6.1[a]	143.2 ± 5.0[a]	1.80 ± 0.01[a]	2.40 ± 0.05[a]	4.29 ± 0.18[a]	3.15 ± 0.12[a]	7.29 ± 0.21[a]	3.83 ± 0.16[a]
IV	389.6 ± 8.0[b]	210.1 ± 5.8[b]	210.5 ± 4.7[b]	3.52 ± 0.04[b]	6.01 ± 0.07[b]	6.98 ± 0.25[b]	6.25 ± 0.16[b]	10.68 ± 0.25[b]	7.23 ± 0.11[b]
V	265.5 ± 7.6[c]	182.6 ± 6.1[c]	186.4 ± 4.6[c]	2.50 ± 0.03[c]	4.28 ± 0.06[c]	5.25 ± 0.21[c]	5.24 ± 0.09[c]	9.25 ± 0.24[c]	5.24 ± 0.15[c]
VI	182.4 ± 7.5[a]	131.2 ± 6.0[c]	151.9 ± 5.1[a]	1.91 ± 0.01[a]	2.54 ± 0.02[a]	4.39 ± 0.18[a]	3.31 ± 0.11[a]	7.38 ± 0.21[a]	3.75 ± 0.19[a]
VII	256.9 ± 8.1[c]	173.2 ± 5.7[c]	187.6 ± 5.8[c]	2.34 ± 0.02[c]	4.58 ± 0.03[c]	5.36 ± 0.17[c]	4.69 ± 0.15[c]	9.12 ± 0.24[c]	5.35 ± 0.07[c]
VIII	272.8 ± 7.9[c]	165.8 ± 5.6[c]	171.2 ± 4.5[c]	2.01 ± 0.01[c]	4.01 ± 0.05[c]	5.01 ± 0.18[c]	4.01 ± 0.12[c]	8.95 ± 0.21[c]	4.69 ± 0.11[c]

Values are mean ± SD for 10 rats. Values with different superscripts differ significantly ($P < 0.05$), SOD: unit = 50% inhibition of NBT reduction, CAT: unit = μmoles of H_2O_2 decomposed, GPx: unit = μmoles of NADPH oxidized

I control, *II* C + CFP (100 mg), *III* C + CFP (200 mg), *IV* diabetic (D), *V* D + CFP (100 mg), *VI* D + CFP (200 mg), *VII* D + Tolbutamide (100 mg), *VIII* D + Metformin (10 mg)

[a] Significantly ($P < 0.05$) different from diabetic rats

[b] Significantly ($P < 0.05$) different from normal rats

[c] Significantly ($P < 0.05$) different from normal and diabetic rats

generation and oxidative stress are hypothesised to play an important role in the pathogenesis of diabetes and its later complications [24]. Diabetic state is shown to be associated with depletion of antioxidants [25]. Vucic et al. [26] reported that the activity of SOD is low in diabetes mellitus.

Decreased catalase activity in plasma and tissues of streptozotocin diabetic rats, may be due to increased utilization to scavenge the toxic products of lipid peroxidation or decreased availability of H_2O_2, the substrate for catalase.

GPx functions as a free radical scavenger and repairs the free radical induced biological damage. The activity of GPx was decreased in plasma, liver and kidney of streptozotocin-induced diabetic rats [27]. The activities of enzymes involved in maintaining normal GSH levels also have been found to be abnormal in tissues during hyperglycemia. The depletion of GSH content may also lower the GPx activity, as GSH is required as substrate for GPx activity [28].

Administration of both concentrations of CFP significantly ($P < 0.05$) increased the activities of enzymatic antioxidants SOD, CAT and GPx.

Dietary supplementation of antioxidants can mitigate the peroxidation reactions and oxidative stress in diabetic animals. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases [29].

CFP showed a protective effect against free radical damage. Administration of CFP tends to bring back the activities of antioxidant enzymes to normal levels. The antioxidant effect of CFP was found to be more prominent when compared to tolbutamide and metformin. CFP at the concentration of 200 mg was found to be more effective than 100 mg of CFP.

The results imply the antiperoxidative activity of CFP, which could exert a beneficial action against pathological alterations produced by the presence of $O^{\bullet -}$ and $^{\bullet}OH$ in tissues. This effective antiperoxidative activity of CFP may prevent the cardiovascular complications that may arise due to lipid peroxidation in cardiac tissues, especially in diabetic conditions [30].

Changes in the Level of Non Enzymic Antioxidants in Diabetic Experimental Rats

Table 3 shows represents the levels vitamin C, vitamin E and reduced glutathione In control rats treated with CFP 100 mg and 200 mg/kg bw activities of the antioxidant enzymes were not significantly altered in plasma, liver and kidney.

There was no significant change recorded in the levels of vitamin C, vitamin E and reduced glutathione in the control rats and those control rats treated with both concentrations of CFP.

The plasma level of ascorbic acid was significantly ($P < 0.05$) higher in diabetic rats than in control rats and those, treated with both concentrations of CFP. This might be due to impaired uptake of ascorbic acid by cells. Hypoinsulinemia and/hyperglycemia inhibits ascorbic acid transport [31]. As the chemical structure of ascorbic acid is similar to that of glucose, it shares the membrane transport system with glucose and hence competes with it for its transport. Ascorbate also acts as a prooxidant at a higher concentration. In such a condition, ascorbate results in increased lipid peroxidation products [32].

Table 3 Effect of CFP on the level of vitamin C, vitamin E and reduced glutathione

Groups	Ascorbic acid			Vitamin E			Reduced glutathione		
	Plasma mg/dl)	Liver (mg/g)	Kidney (mg/g)	Plasma (mg/dl)	Liver (mg/g)	Kidney (mg/g)	Plasma (mole/dl)	Liver (nmole/g)	Kidney (nmole/g)
I	0.28 ± 0.02^{a}	0.07 ± 0.02^{a}	0.06 ± 0.011^{a}	0.18 ± 0.02^{a}	0.41 ± 0.04^{a}	0.36 ± 0.04^{a}	6.74 ± 0.12^{a}	8.65 ± 0.19^{a}	6.86 ± 0.14^{a}
II	0.27 ± 0.04^{a}	0.08 ± 0.02^{a}	0.07 ± 0.013^{a}	0.19 ± 0.02^{a}	0.42 ± 0.04^{a}	0.35 ± 0.04^{a}	6.79 ± 0.11^{a}	8.55 ± 0.14^{a}	6.87 ± 0.12^{a}
III	0.28 ± 0.03^{a}	0.07 ± 0.01^{a}	0.07 ± 0.010^{a}	0.20 ± 0.03^{a}	0.43 ± 0.05^{a}	0.34 ± 0.04^{a}	6.72 ± 0.09^{a}	8.61 ± 0.15^{a}	6.91 ± 0.11^{a}
IV	0.66 ± 0.04^{b}	0.04 ± 0.01^{d}	0.03 ± 0.010^{b}	0.07 ± 0.02^{b}	0.20 ± 0.04^{b}	0.12 ± 0.03^{b}	5.17 ± 0.14^{b}	4.98 ± 0.17^{b}	4.42 ± 0.09^{b}
V	0.27 ± 0.03^{a}	0.05 ± 0.01^{c}	0.04 ± 0.012^{c}	0.13 ± 0.03^{a}	0.34 ± 0.05^{b}	0.25 ± 0.06^{c}	5.82 ± 0.09^{c}	6.98 ± 0.16^{c}	6.05 ± 0.12^{c}
VI	0.28 ± 0.03^{a}	0.08 ± 0.02^{a}	0.06 ± 0.011^{a}	0.17 ± 0.03^{a}	0.43 ± 0.05^{a}	0.37 ± 0.04^{a}	6.59 ± 0.08^{a}	8.45 ± 0.18^{a}	6.90 ± 0.13^{a}
VII	0.28 ± 0.02^{a}	0.04 ± 0.01^{c}	0.03 ± 0.013^{c}	0.09 ± 0.03^{b}	0.25 ± 0.04^{c}	0.19 ± 0.03^{c}	5.87 ± 0.12^{c}	5.63 ± 0.17^{c}	5.92 ± 0.15^{c}
VIII	0.28 ± 0.02^{a}	0.07 ± 0.02^{a}	0.06 ± 0.011^{a}	0.18 ± 0.02^{a}	0.41 ± 0.04^{a}	0.36 ± 0.04^{a}	5.98 ± 0.09^{c}	6.54 ± 0.14^{c}	5.39 ± 0.13^{c}

Values are mean ± SD for 10 rats. Values with different superscripts differ significantly ($P < 0.05$)

I control, *II* C + CFP (100 mg), *III* C + CFP (200 mg), *IV* diabetic (D), *V* D + CFP (100 mg), *VI* D + CFP (200 mg), *VII* D + Tolbutamide (100 mg), *VIII* D + Metformin (10 mg)

[a] significantly ($P < 0.05$) different from diabetic rats

[b] significantly ($P < 0.05$) different from normal rats

[c] significantly ($P < 0.05$) different from normal and diabetic rats

Significantly ($P < 0.05$) lower levels of vitamin E and reduced glutathione were observed in diabetic control rats. It represents the increased utilization of these antioxidants to counteract the oxidative stress in the diabetic state [33].

The levels of ascorbic acid, α-tocopherol and reduced glutathione were significantly ($P < 0.05$) decreased in liver and kidney of diabetic rats as compared to the various controls. These antioxidants exist in interconvertable (reduced and oxidized) forms. Thus, reduction in the level of these antioxidants in the diabetic rats is attributed to the reduced regeneration from their oxidized forms [34].

Administration of both the concentrations of CFP to streptozotocin-induced diabetic rats prevented a significant increase in ascorbic acid level and significant decrease in α-tocopherol and reduced glutathione in plasma, liver and kidney. Administration of CFP brought about a decrease in ascorbic acid in plasma. On the contrary it is increased in liver and kidney. The other two non-enzymic antioxidants, α-tocopherol and reduced glutathione were increased both in plasma and tissues, which will eventually reduce peroxidation. Both the concentrations of CFP were found to be better than tolbutamide and metformin in this respect. In comparison with 100 mg, 200 mg of CFP induced better antioxidant effect.

Conclusion

Studies on antioxidant action demonstrated that the concentrations of malondialdehyde, hydroperoxides and conjugated dienes in serum, liver and kidney of anti diabetic herbal drug CFP treated diabetic rats showed significant reduction indicating the increased scavenging of lipid peroxides or decreased rate of lipid peroxidation. Thus both the concentrations of CFP exhibit highly significant inhibitory effects of lipid oxidation. The end products of lipid peroxidation are known to induce cellular damage and free radical induced diseases like diabetes mellitus and the secondary complications like cataract, retinopathy and cardiovascular disorders. These can be prevented by CFP administration.

A concomitant increase in the antiperoxidative enzymes namely catalase, glutathione peroxidase and superoxide dismutase in serum, liver and kidney of CFP treated diabetic rats was observed. Also, an increase in the non-enzymic antioxidants, vitamin E and reduced glutathione and decrease in ascorbic acid was observed. This indicates that these herbal powders play an important role in scavenging toxic intermediates of incomplete oxidation in the body. Antiperoxidative effect of 200 mg CFP was much more significant than that of 100 mg of CFP.

References

1. Vendemiale G, Grattagliano I, Altomare E. An update on the role of free radicals and antioxidant defense in human diseases. Int J Clin Lab Res. 1999;29(2):40–5.
2. Halliwell B. Free radicals, antioxidant and human disease, curiosity, cause or consequence? Lancet. 1994;344:721–4.
3. De Leiva A, Lefebvre P, Nerup J. European dimension of diabetes research. Dibetologia. 1995;35:5–11.
4. Ceriello A, Bortolotti N, Falleti E, Taboga C, Tonutti L, Crescentinoi A, Motz Lizzio S, Russo A, Bartoli E. Total radical-trapping antioxidant parameter in NIDDM patients. Diabetes Care. 1997;20:194–7.

5. Maejima K, Nakano S, Himeno M, Tsuda S, Makiishi H, Ito T, Nakagawa A, Kogoshi T, Nishio M, Uchida K. Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. J Diabetes Complicat. 2001;15:135–43.
6. Murugesan MCS. In: Gunapadam, vegetable section, 3rd ed. 1980. p. 65
7. Nichans WJ Jr, Samuelson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem. 1968;6:126.
8. Mair RD, Hall T. In: Swem, DC, Witky, editors. Organic peroxide, vol. 2. New York: Intersciences, Wikyced; 1977. p. 532.
9. Recknagel RO, Ghoshal AK. New data on the question of lipoperoxidation in carbon tetrachloride poisoning. Ex Mol Pathol. 1966;5:43.
10. Kakkar P, Das B, Viswanathan PNA. Modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21:30–132.
11. Sinha KA. Colorimetric assay of catalase. Ann Biochem. 1972;47:389–94.
12. Rotruck JT, Pope AL, Ganther HE, Swanson AB. Biochemical roles as a component of glutathione peroxidase. Science. 1984; 179:588–90.
13. Moron MS, De Pierre JN, Mannervik V. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat lung and liver. Biochem Biophys Acta. 1979;582:67–8.
14. Omaye ST, Turbul TD, Sauberlich HC. Selected method for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCormic DB, Wright DL, editors. Methods of Enzymology, vol. 62. Academic Press: New York; 1979. pp. 3–11.
15. Chakarabarti R, Vikaramadithyan RK, Mullangi R, Sharma VM, Jugedheshan H, Rao YN. Antidiabetic and hypolipidemic activity of *Helicteres isora* in animal models. J Ethnopharmacol. 1982; 81(3):343–9.
16. Bhavapriya V, Kalpana S, Govindasmy S, Apparanantham T. Biochemical studies on hypoglycemic effect of *Aavirai kudineer:* a herbal formulation in alloxan diabetic rats. Indian J Exp Biol. 2001;39:925–8.
17. Garg MC, Bansal DD. Protective antioxidant effect of vitamins C and E in steptozotocin induced diabetic rats. Indian J Exp Biol. 2000;38:101–4.
18. Pari L, Latha M. Effect of *Cassia auriculata* flowers on blood sugar levels serum and tissue lipids in streptozotocin diabetic rats. Singap Med J. 2002;43(12):617–21.
19. Maxwell SRJ, Thomson H, Sandler D, Leguan C, Baxlex AM, Thorpe GHG, Jones AF, Barnett AH. Poor glycemic control is associated with reduced serum free radical scavenging (antioxidant) activity in non-insulin-dependent diabetes mellitus. Ann Clin Biochem. 1997;27:638–44.
20. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G. Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. Diabetes. 1997;46:1853–8.
21. Steer KA, Wallace TM, Bolton CH, Hartog M. Aspirin protects low-density lipoprotein from oxidative modification. Heart. 1997; 77:333.
22. Bassirat M, Khalil Z. Endothelin and free radicals modulate microvascular responses in steptozotocin-induced diabetic rats. Microvas Res. 2000;59(1):88–9.
23. Elizabeth GHJ. Human cytochrome P_{450} enzymes expressed in bacteria: reagent to probe molecular interaction in toxicology. Clin Exp Pharmacol Physiol. 1998;25:877–86.
24. Vijayalingam S. Abnormal antioxidant status in impaired glucose tolerance in non-insulin dependent diabetes mellitus. Diabet Med. 1996;13(8):175–9.
25. Kumari K, Augusti KT. Antioxidant and antocarcinogenic activity of lucovin-an indigenous herbal preparation, Indian. J Exp Biol. 1995;37:1177–81.
26. Vucic M, Gavell M, Bozikov V, Ashcroft JH, Rocic B. Superoxide dismutase activity in lymphocytes and polymorphonuclear cells of diabetic patients. Eur J Clin Chem Clin Biochem. 1997; 266:6068.
27. Raza H, Ahmed I, John A, Sharma AK. Modulation of xenobiotic metabolism and oxidative stress in chronic streptozotocin diabetic rats fed with *Momordica charantia* fruit extract. J Biochem Mol Toxicol. 2000;14(3):131–9.
28. Y-Usong Y, Ji-Zhong C, Sharad S, Yogesh C. Role of GST in protection against lipid peroxidation. J Biol Chem. 2001;276: 1920–30.
29. Malencic DJ, Gasic O, Popovie M, Boza P. Screening antioxidant properties of *Salriareflexa horem*. Phytother Res. 2000;14:546–8.
30. Krishnakumar K, Augusti KT, Vijayammal PL. Hypoglycemic and antioxidant activity of *Saacic oblonga wall* extract in steptozotocin induced diabetic rats. Indian J Physiol Pharmacol. 1997;43:510–4.
31. Mann GV, Newton P. The transport of ascorbic acid. Ann NY Acad Sci. 1975;258:243.
32. Halliwell B. Vitamin C, antioxidant and peroxidant *invivo*. Free Rad Res Comm. 1997;25:439–54.
33. Ceriello A, Gangliano D, Quatraro A, Donzella C, Dipalo G, Lefevre P. Vitamin E reduction of antiglycosylation in diabetes, new prospects for prevention of diabetic complications. Diabet Med. 1991;9:297–9.
34. Anuradha CV, Ravikumar P. Restoration of tissue anti oxidants by fenugreek seeds (*Trigonella foenum graceum*) in alloxan-diabetic rats. Indian J Physiol Pharmacol. 2001;45(4):408–20.