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Free Radical Scavenging Activity of Methanolic Leaf Extract of *Bombax Ceiba* on Streptozotocin Induced Diabetic Rats

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ABSTRACT

The main objective of the study is to verify the antioxidant activity of methanolic leaf extract of *Bombax ceiba* by evaluating the superoxide dismutase, catalase and lipid peroxidation. Diabetes was induced experimentally in 12-hr-fasted rats by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 115 mg/kg. Blood samples were withdrawn from the tail vein at intervals of 30, 60, 90 and 120 min of glucose administration. The results showed that the antioxidant enzymes such as CAT, SOD and GPX were significantly ($p < 0.05$) decreased in diabetic rats compared to normal rats and extract treated rats exhibited significantly increased in these levels of kidney, liver and pancreas tissues respectively than untreated diabetic rats, where as inversely lipid peroxidation was lowered. The mean levels of vitamins C and E were found to be significantly increased in methanolic leaf extract of *Bombax ceiba* -treated diabetic rats. It may be suggested that methanolic leaf extract of *Bombax ceiba* confers protection against experimental diabetes through its anti hyperglycemic and antioxidant properties.

Keywords: Streptozotocin (STZ), Antioxidant, *Bombax ceiba*, SOD, Catalase, Lipid Peroxidation.

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1. Introduction

Antioxidants are chief line defense against free radical damage and critical for maintain optimum health and well being. Hyperglycemia impairs the prooxidant/antioxidant balance, higher free radicals and reducing antioxidant levels. Free radicals react with lipids and causes peroxidative change that result in improved lipid peroxidation. The level of lipid peroxidation in cells is controlled by different cellular defense mechanisms. The higher free radical production exerts cytotoxic effects on the membrane phospholipid, resulting in the formation of toxic products such as MDA. The antioxidant scavenging enzymes SOD and GSH-Px offer protection to cells and tissues against oxidative injury (WHO 2006). Some herbal drugs are a good source of natural antioxidants. *Bombax ceiba* is a medicinal tree and is also referred as silent doctor. It is found in India, Malaysia, Sri lanka, Hong kong, Australia and Africa. Every part of this tree is used to treat various ailments. In Ayurveda it is referred as aphrodisiac, astringent, antidiarrheal, antidysenteric, antimicrobial diuretic, alterative, antipyretic and tonic. It is used in treatment of asthma, diarrhoea, wound, leucorrhoea, anaemia, seminal disorders and skin problems. Therefore to verify the antioxidant activities of methanolic extract of *Bombax ceiba* leaves by evaluate antioxidant enzymes such as Superoxide dismutase, catalase and lipid peroxidation.

2. Material and methods

Plant Extraction

The leaves of *B. ceiba* (Illavam panju) was collected and dried. The leaves are dried in shade for more than 1 week. The leaf materials were dried under shade and grinded to coarse powder. Powdered leaf materials (50g) were extracted with methanol (500ml) and then filtered. The methanolic extract of *Bombax ceiba* leaf powder was collected by using Soxhlet apparatus.

Experimental Animals

Male albino Wister rats (25) were brought for the study. The animals were fed with standard laboratory food pellet and tap water was available *ad libitum*. The animals were housed in polypyrrolene cages, i.e. five in single cage likewise in five cages. They maintained in the controlled temperature room (22°C), with a 12h light: 12h dark cycle.

Experimental Induction of Diabetes Mellitus

Diabetes was induced experimentally in 12-h-fasted rats by a single *ip* injection of STZ (115 mg/kg) dissolved in 0.1 M of cold citrate buffer (pH 4.5) (Marles *et al.*, 1995). Since STZ is capable of inducing hypoglycemia due to marked release of insulin from the pancreas, the STZ-administered rats were provided after 6 h with a 10% glucose solution for 24 h so as to prevent hypoglycemia. After 72 h, rats with a blood glucose concentration above 250 mg/dl were considered to be diabetic.

Grouping of Animals

25 rats were grouped into five groups and each group contains five rats.

Group 1:

(Normal Control) animals received 1% tween 80 (5ml/kg, p.o) once daily

Group 2: STZ induced diabetic rats received methanolic extract of *Bombax ceiba* leaves, 250mg/kg, p.o. suspended in 1% tween 80

Group 3: Methanolic extract of *Bombax ceiba* leaves, 300mg/kg, p.o. suspended in 1% tween 80

Group 4: STZ induced diabetic rats received only vehicle 1% tween 80 (5ml/kg, p.o)

Group 5: STZ induced diabetic rats received Glibenclamide (600 µg/kg p.o) suspended in 1% tween 80

Preparation of tissue homogenates

The rats were sacrificed by cervical dislocation and tissues (liver, kidney and pancreas) were excised, rinsed in ice-cold physiological saline, and homogenized with Potter Elvehjem homogenizer. 10% homogenates were prepared in known volume of certain buffer and centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was used for antioxidant enzyme assays superoxide dismutase (SOD, CAT, GSH and lipid peroxidation).

Enzymatic assays

Assay of superoxide dismutase (SOD)

SOD was assayed according to the method of Kakkar *et al.*, 1984.

Assay of catalase (CAT)

Catalase activity was assayed following the method of Luck *et al.*, 1965.

Estimation of reduced glutathione (GSH)

Reduced glutathione was determined by the method of Moron *et al.*, 1979.

Assay of Malondialdehyde Lipid Peroxidation

The extent of lipid peroxidation was estimated according to the method of Okhawa *et al.*, 1979.

Assay of Glutathione Peroxidase

Glutathione peroxidase was determined by the method of Rotruck *et al.*, 1973.

3. Results and Discussion

Activities of enzymatic antioxidants in liver, kidney and pancreatic tissue of Wistar rats

There were no significant differences between the mean activities of SOD, CAT and Gpx in the liver, kidney and pancreatic tissue of normal methanolic leaf extract of *Bombax ceiba* -treated (Group III) rats and the mean activities in liver, kidney and pancreatic tissue of normal untreated (Group I) rats. Significantly (P<0.05) subordinate mean activities of these enzymatic antioxidants were observed in the liver, kidney and pancreatic tissue of diabetic untreated (Group IV) rats than those in Group I and Group III rats. However, significantly (P<0.05) increased mean activities of these enzymes were observed in diabetic rats treated with methanolic leaf extract of *Bombax ceiba* (Group II) and diabetic rats treated with glyclazide (Group V) than those in Group IV rats. There were no significant differences in the mean values of these parameters between methanolic leaf extract of *Bombax ceiba* -treated diabetic (Group II) rats and glyclazide-treated diabetic (Group V) rats; however, the mean values of CAT and G_{px} in Group IV and Group V rats were significantly lower than those in untreated normal methanolic leaf extract of *Bombax ceiba* normal rats (Tables 1-3).

Concentrations of non-enzymatic antioxidants in liver, kidney and pancreatic tissue of Wistar rats

The mean levels of GSH in the liver, kidney and pancreatic tissue samples of diabetic untreated rats were significantly ($P < 0.05$) elevated than those in normal untreated and normal methanolic leaf extract of *Bombax ceiba* -treated rats. In diabetic rats treated with methanolic leaf extract of *Bombax ceiba* and those treated with glyclazide showed significantly ($P < 0.05$) higher mean levels of GSH when compared to the diabetic untreated rats; hence, they remained significantly lower than those in normal untreated and normal methanolic leaf extract of *Bombax ceiba* -treated rats. No significant differences in the mean values of these parameters were noted between diabetic methanolic leaf extract of *Bombax ceiba* -treated rats and diabetic glyclazide-treated rats (Fig. 4).

Malondialdehyde (MDA) concentrations in liver, kidney and pancreatic tissues of Wistar rats

There was no significant difference between the mean values of liver, kidney and pancreatic tissue MDA in Group III (normal methanolic leaf extract of *Bombax ceiba* -treated) rats and Group I (normal untreated) rats. The mean concentration of MDA in the liver, kidney and pancreatic tissue of Group IV (diabetic untreated) rats was significantly ($P < 0.05$) increased than that in Group I (normal untreated) and Group III (normal methanolic leaf extract of *Bombax ceiba* treated) rats. While the mean levels of liver, kidney and pancreatic tissue MDA in diabetic rats treated with methanolic leaf extract of *Bombax ceiba* (Group II) and in those treated with glyclazide (Group V) were significantly ($P < 0.05$) decrease than that in diabetic untreated (Group IV) rats, these levels were not significantly different to those in normal untreated (Group I) and normal methanolic leaf extract of *Bombax ceiba* treated (Group III) rats (Fig. 5).

Table 1: Activity of enzymatic antioxidant levels of SOD in liver, kidney and pancreatic tissue of Wistar rats

Groups	SOD		
	Kidney	Liver	Pancreas
Normal control ^{*a}	186±3.54	194±8.12	179±6.95
Diabetes + Extract ^{*ab}	206.4±11.46	200.3±6.27	211.4±7.75
Normal + Extract ^{*b}	236.8±44.09	247±8.19	213.2±5.72
Diabetic control ^{*b}	275.1±10.87	292±3.59	299.1±8.38
Positive control ^{*b}	188.6±5.37	189.9±7.93	175.4±10.80

Values are expressed as mean ± SEM; * $P < 0.05$

a is used to indicate the significance between Group II Vs Group I

b is used to indicate the significance between Group II Vs Group IV & V.

Data were analyzed by One-way ANOVA.

Table 2: Catalase activity in liver, kidney and pancreatic tissue of control, diabetic and methanolic leaf extract of *B. ceiba* treated rats.

Groups	Catalase		
	Kidney	Liver	Pancreas
Normal control ^{*a}	15.758±0.41	15.11±0.954	14.99±0.79
Diabetes + Extract ^{*ab}	14.218±0.64	13.91±0.753	14.821±0.79
Normal + Extract ^{*b}	13.942±0.89	14.32±0.728	14.234±0.81
Diabetic control ^{*b}	10.491±0.50	9.59±1.384	10.763±0.53
Positive control ^{*b}	14.457±0.56	14.09±0.801	14.81±0.20

Values are expressed as mean ± SEM; * $P < 0.05$

a is used to indicate the significance between Group II Vs Group I

b is used to indicate the significance between Group II Vs Group IV & V.

Data were analyzed by One-way ANOVA.

Table 3: Estimation of Glutathione peroxidase in liver, kidney and pancreatic tissue of control, diabetic and methanolic leaf extract of *B. ceiba* treated rats.

Groups	Glutathione peroxidase		
	Kidney	Liver	Pancreas
Normal control ^{*a}	34.06±1.23	34.9±1.70	34.5±0.79
Diabetes + Extract ^{*ab}	36.54±0.38	37±0.99	36.49±0.79
Normal + Extract ^{*b}	35.836±0.34	35.99±0.89	35.23±0.81
Diabetic control ^{*b}	28.009±1.45	28±1.50	29.5±0.53
Positive control ^{*b}	31.619±1.34	29.9±0.97	31±0.20

Values are expressed as mean ± SEM; * $P < 0.05$

a is used to indicate the significance between Group II Vs Group I

b is used to indicate the significance between Group II Vs Group IV & V.

Data were analyzed by One-way ANOVA.

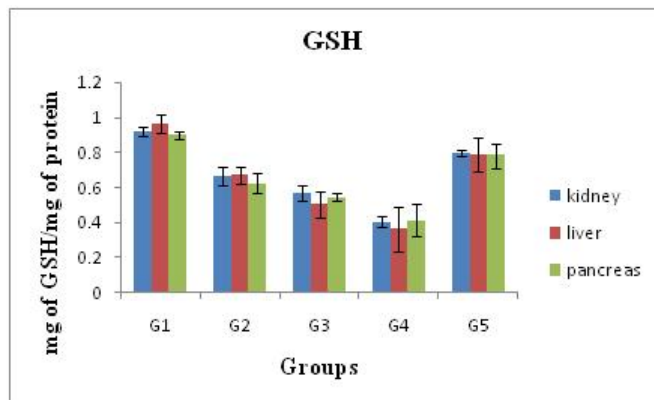


Figure 4: Changes of GSH in liver, kidney and pancreatic tissue of control, diabetic and methanolic leaf extract of *B. ceiba* treated rats.

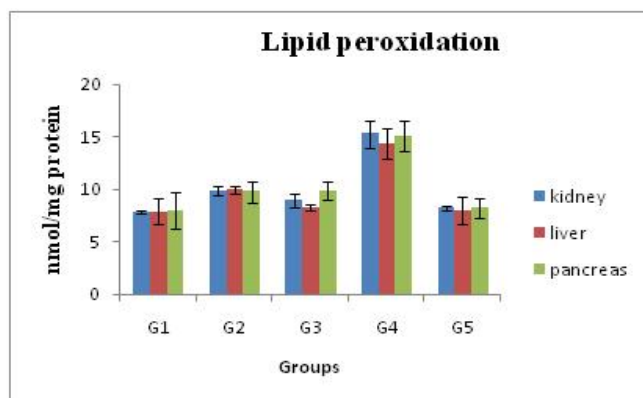


Figure 5: Changes of Lipid peroxidase in liver, kidney and pancreatic tissue of control, diabetic and methanolic leaf extract of *B. ceiba* treated rats.

Discussion

The mean activity of Superoxide dismutase, Catalase, GPx, and GSH in liver, pancreas and kidney tissues in group IV rats were found to be significantly lower than that of group I, II, III and V rats. However, a group II rat was found to be similar to that of group I rats. Moreover, a significant result was placed in group III and V rats that was about equal to that of group I. The mean activity of Lipid peroxidation in liver, pancreas and kidney tissues in group IV rats were found to be significantly higher than that of group I, II, III and V rats. However, a group II rat was found to be similar to that of group I rats. Moreover, a significant result was placed in group III and V rats that was about equal to that of group I. The chronic hyperglycemia occurring in diabetes mellitus might direct to an increased fabrication of reactive oxygen species (ROS), this probably resulting in depletion of certain non-enzymatic or enzymatic scavengers. Superoxide dismutase (SOD) is an innate cellular antioxidant enzyme which catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2), though catalase (CAT) is a hemeprotein which converts H_2O_2 to water and oxygen⁸. Glutathione peroxidase (Gpx), being a selenium-containing enzyme, aids the elimination of H_2O_2 , there in preventing the formation of hydroxyl radical $[OH\cdot]$; it also degrades hydroperoxide, a potentially toxic molecule. In the current

study, the mean activities of SOD, CAT and Gpx in liver, kidney and pancreatic tissue samples were found to be significantly decreased in diabetic untreated rats than in untreated normal rats. The decrease mean activity of SOD was probably due to accumulated superoxide radicals, H_2O_2 -mediated deactivation of SOD or glycosylation of SOD. Similar mechanisms probably accounted for the decrease mean CAT activity as well (Mates JM *et al* 1999). The lower mean Gpx activity observed in liver, kidney and pancreatic tissue of diabetic untreated rats may be represented vital adaptive response to higher peroxidative stress, as hypothesized by Kinalski *et al*. Hence, in the current investigation, significantly ($P < 0.05$) increased mean activities of SOD, CAT and Gpx were noted in the liver, kidney and pancreatic tissue of methanolic leaf extract of *Bombax ceiba* treated diabetic rats than in diabetic untreated rats. Reduced glutathione (GSH) is a major non-protein thiol in living organisms. Thiol groups are supposed to play the vital role in maintaining the intracellular and membrane redox state of the secretory function of -pancreatic cells (Mates JM *et al* 1999).

In the current study, the decreased mean levels of GSH in untreated diabetic rats were probably due to more consumption of GSH as an antioxidant defense against ROS. However, methanolic leaf extract of *Bombax ceiba* treated diabetic rats exhibited significantly increased mean levels of GSH than untreated diabetic rats. Methanolic leaf extract of *Bombax ceiba* might act by dipping hyperglycemia-mediated oxidative stress maybe reduces the consumption of free radical scavengers. Lipid peroxidation, a free-radical mediated dissemination of oxidative abuse to polyunsaturated fatty acids, is a distinctive feature of chronic diabetes; it impairs cell membrane fluidity and modifies the activity of membrane-bound enzymes and receptors, ensuing in membrane malfunction (Kinalski M *et al* 2000). Malondialdehyde (MDA), a secondary product of lipid peroxidation, is an indicator of tissue damage. The elevated mean level of MDA in diabetic untreated (Group III) rats in the current investigation probably resulted from higher intensity of lipid peroxidation occurring due to intensified free radical production, as suggested earlier (Paolisso G *et al* 1993). Oral administration of methanolic leaf extract of *Bombax ceiba* to diabetic rats brought about a significant decrease in MDA. methanolic leaf extract of *Bombax ceiba* probably forage free radicals, therein stabilizing the endogenous antioxidant defense network and lower the lipid peroxidation, such as quercetin (Halliwell B *et al* 2000). The methanolic leaf extract of *Bombax ceiba* possibly protected the pancreatic islets from free radical-mediated oxidative stress and conserved the integrity of pancreatic -cells, thereby stimulating the remaining pancreatic -cells to synthesize and exude additional insulin to maintain glucose homeostasis. Such a sequence of events has been proposed by other investigators to bolster the antioxidant defense system. There are reports suggesting that enzymatic antioxidants such CAT, SOD prevent the damage to isolated pancreatic cells exposed to the diabetogenic agent (Maritim AC *et al* 2003). Asplundh *et al* (1984) found that SOD coupled to polyethylene glycol

reduced the hyperglycemic response in STZ induced rats. Evaluation of the endogenous enzymatic & non-enzymatic antioxidants in the hepatic and pancreatic tissues for all the groups indicates the possible evidence for the antihyperglycemic activity of the *Bombax ceiba*.

The findings of our study are in agreement with other studies which reported increased levels of lipid peroxidation in pancreas of diabetic rats after treatment with *Bombax ceiba*. Particularly in pancreatic cells, increased stress level in the form of lipid peroxidation, Vitamin C and E can alter the structure of cell membrane lipids and compromising the cell viability (Grankvist K et al 1979). The results showed that the antioxidant enzymes such as CAT, SOD were significantly ($p < 0.05$) decreased in diabetic rats compared to normal rats in kidney, liver and pancreas tissues respectively as shown in figures. Similarly, the non-enzymatic antioxidants such as GSH was significantly lower whereas MDA levels increased in untreated diabetic rats compared to normal rats. Diabetic groups rat treated with Methanolic extract of *Bombax ceiba* exhibited significantly higher ($p < 0.05$) CAT enzyme level. Similarly, the SOD levels were also elevated significantly ($p < 0.05$) in the liver, kidney and pancreas homogenates compared to diabetic rats. Diabetic treated group animals showed increased levels of liver, kidney and pancreas antioxidant enzyme G_{px} as compared to diabetic rats. Treatment with *Bombax ceiba* combats the decreased levels of these enzymes, thereby indicating the protective effect of *Bombax ceiba*. Reduced glutathione play a significant role in the generation of cellular redox state and consequently, the imbalance in reduced glutathione to oxidized glutathione ratio is a putative indicator of cellular oxidative stress. Our investigation indicated that, *Bombax ceiba* possessed antihyperglycemic activity as well as protective effect against lipid peroxidation.

4. Conclusion

In conclusion, it may be suggested that methanolic leaf extract of *Bombax ceiba* confers protection against experimental diabetes through its antihyperglycemic and antioxidant properties.

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