



ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF BUTEA FRONDOSA LEAVES WITH ITS POSSIBLE MECHANISM OF ACTION

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ABSTRACT

Purpose: The aim of the present study was to investigate antidiabetic activity of methanolic extract of *Butea frondosa* leaves with its antioxidant potential

Method: Effect of methanolic extract of *Butea frondosa* leaves at a dose level of 100mg/kg and 200mg/kg on blood glucose, haemoglobin, serum total protein, serum total cholesterol, HDL cholesterol, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and also for the levels of antioxidant enzyme such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and lipid peroxidation (LPO) in pancreatic homogenate was studied on alloxan induced diabetic rats

Result: Methanolic extract of *Butea frondosa* leaves at dose of 100 mg/kg and 200 mg/kg treated groups showed considerable improvement in all the parameters studied in a dose dependant manner. Histopathological studies also revealed that treatment by methanolic extract of *Butea frondosa* reduced pancreatic damage caused by alloxan.

Conclusion: Antihyperglycaemic action of methanolic extract of *Butea frondosa* leaves may be due to antioxidant potential of extract which is revealed by improvement in the levels of antioxidant enzymes in pancreas of alloxan diabetic rats and validate its claim in Indian system of medicine.

Keywords: *Butea frondosa*, antidiabetic, antioxidant enzyme, alloxan.

INTRODUCTION

Diabetes mellitus is a major global problem of considerable magnitude till date as significant percentage of human population is affected by the disease. Despite considerable progress in the management of diabetes mellitus, with insulin therapy, oral hypoglycemic agents, restricted diet, exercise either singly or in combination, the search for indigenous antidiabetic agent still continues, as a large number of cases treated with traditional medicine in the form of plant extract have been reported to give remarkably good results.

In diabetes there is increased oxidative stress in combination with reduced antioxidant status, this result in greater vulnerability to the damaging effects of free radical. Oxidative free radical - induced degeneration of pancreatic beta islet cells has been implicated in the etiopathogenesis of clinical diabetes mellitus. Several enzyme system present in our body glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) can scavenge reactive oxygen species following different mechanism. The plant *Butea frondosa* (B. frondosa; Family: Leguminosae) grows throughout India ¹ and has been extensively studied for its anthelmintic ², antifertility ³ and hepatoprotective activities⁴. The traditional system of medicine claims that leaves of *Butea frondosa* are used in inflammatory condition, liver disease, skin disease, worm infestation, haemorrhoids and in diabetes ⁵. The current investigation has been designed to evaluate antidiabetic activity methanolic extract of *Butea frondosa* leaves with its antioxidant potential.

MATERIALS AND METHODS

Plant material

The plant material of *Butea frondosa* leaves used for investigation was collected from Pathanamthitta district of Kerala and the leaves of *Butea frondosa* was identified and authenticated by Dr. Sasikala Ethirajulu, research officer (pharmacognosy) Central Research Institute (Siddha), Chennai and voucher specimen of the plant has been deposited in the herbarium of the department.

Preparation of extracts

The leaves of *Butea frondosa* were shade-dried and powdered to a coarse powder, and the powder was passed through a 40-mesh sieve and were subjected to continuous hot extraction in Soxhlet

apparatus with petroleum ether (60% v/v) and the marc left after petroleum ether extraction were dried and extracted with methanol (90% v/v) in Soxhlet apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent had been removed to give an extract sample with a yield of 8.5% w/w. Methanolic extract of *Butea frondosa* (MEBF) was used for pharmacological studies.

Animals

Albino wistar rats of either sex approximately same age group having weight 150- 200g were used after being acclimatized for a week at laboratory conditions. They were provided standard rodent pellet diet (Lipton India) and water ad libitum. The animals had free access to food and water and maintained under 12:12 hr light and dark cycle. All experiments were carried out during day time from 09.00 to 17.00 hr. The institutional animal ethical committee approved the protocol and care of animals was taken as per guidelines of committee for the purpose of control and supervision in experiments on animals (CPCSEA), representative of animal welfare, Govt of India.

Effect of methanolic extract of *butea frondosa* leaves on alloxan-diabetic rats ⁶

Groups of rats were fasted for 12 hrs and hyperglycemia was induced by injecting intraperitoneally a single dose (150 mg/kg) of 2% alloxan monohydrate solution in saline. 48 hrs after alloxan injection, blood glucose were determined. Rats with blood glucose of 150-350 mg/dl ⁷ were taken for the study. One group consisted of normal rats and received 0.5 ml of vehicle 2% v/v aqueous tween 80 orally, while other alloxan diabetic rats received 0.5 ml of 2% v/v tween 80 as control and standard drug glibenclamide 10 mg/kg orally. Another two alloxan diabetic groups received methanolic extract of *Butea frondosa* leaves at a dose of 100mg and 200mg/Kg orally for 7 days.

All animals were sacrificed under light ether anesthesia, blood was collected and used for various biochemical estimation. Blood glucose level, haemoglobin ⁸, serum total protein ⁹, total cholesterol, HDL cholesterol ¹⁰, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) ¹¹ were estimated. Immediately after sacrifice, the pancreas was dissected out, washed in ice-cold saline and small pieces of pancreatic tissues were

collected in 10% formalin solution for histopathological examination. The histopathological studies were carried by the method described by the Kanai Mukherjee¹². Remaining section of pancreas was used for preparation of 10% homogenate in 0.1M Tris-HCL buffer (pH- 7.4). The homogenate was centrifuged and supernatant was used for assay of superoxide dismutase (SOD)¹³, catalase¹⁴, glutathione peroxidase (GPx)¹⁵, and lipid peroxidation (LPO)¹⁶.

Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnett's 't' test.

RESULTS

Effect of MEBF on alloxan diabetic rats

The antihyperglycemic potential of MEBF was observed. The extract (100 mg/kg & 200 mg/kg p.o.) exhibited significant dose dependent decrease in blood sugar ($p < 0.001$) after seven days of treatment in rats, when compared to alloxan elevated blood glucose level. The standard drug glibenclamide (10 mg/kg p.o.) exhibited significant decrease in blood sugar ($p < 0.001$) at seven days of treatment. Results are shown in Table 1

Table 1: Effect of MEBF after 7 days treatment on blood sugar (mg/dl) level on alloxan induced diabetic rats

Group	Blood Sugar (mg/dl)		
	At the time of grouping	Days of Treatment	
		0 day (48 hrs after alloxan administration)	<i>Butea frondosa</i> Day 7
Control	97.43± 2.99	99.71 ± 4.1	96.14 ± 2.3
Diabetic Control	79.33± 3.88	314.66 ± 7.82	350.83 ± 7.91
Glibenclamide (10mg/kg)	83.66± 6.93	284.33 ± 13.18	98.33 ± 5.05*
MEBF (100 mg/kg)	80.83± 9.20	312.66 ± 20.12	138.5 ± 14.8*
MEBF (200 mg/kg)	98.5 ± 7.76	339.33 ± 14.74	107.6 ± 3.47*

Values are mean ± SEM of 6 rats in each group, * $p < 0.001$ day 7 are compared with 0 day

Effect of MEBF on total protein

The total protein level in serum significantly decreased ($p < 0.001$) in alloxan treated animals when compared to control. Total protein levels of animals treated with MEBF (100 mg/kg & 200 mg/kg p.o.) and glibenclamide (10 mg/kg p.o.) showed significant increase ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 2.

Effect of MEBF on haemoglobin

The haemoglobin level in blood significantly decreased ($p < 0.001$) in alloxan treated animals when compared to control. Total haemoglobin levels of animals treated with MEBF 100mg/kg p.o ($p < 0.01$) & 200 mg/kg p.o. ($p < 0.001$) showed significant increase when compared to alloxan treated animals. Glibenclamide (10 mg/kg p.o.) showed significant increase ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 3.

Effect of MEBF on total cholesterol

The total cholesterol level in serum significantly increased ($p < 0.001$) in alloxan treated animals when compared to control. Total cholesterol levels of animals treated with MEBF (100 mg/kg & 200 mg/kg p.o.) and glibenclamide (10 mg/kg p.o.) showed significant decrease ($p < 0.001$) when compared to alloxan treated animals. Results shown in Table 4.

Effect of MEBF on HDL Cholesterol

The HDL cholesterol level in serum significantly decreased ($p < 0.001$) in alloxan treated animals when compared to control. Total HDL cholesterol levels of animals treated with MEBF 100mg p.o. ($p < 0.05$), 200 mg/kg p.o. ($p < 0.001$) showed significant increase when compared to alloxan treated animals. Glibenclamide (10 mg/kg) showed significant increase ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 5.

Effect of MEBF on Serum Glutamate Oxaloacetate Transaminase

The SGOT level in serum significantly increased ($p < 0.001$) in alloxan treated animals when compared to control. SGOT levels of animals treated with MEBF 100 mg/kg p.o. & 200 mg/kg p.o. and glibenclamide (10 mg/kg p.o.) showed significant decrease ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 6.

Effect of MEBF on Serum Glutamate Pyruvate Transaminase

The SGPT level in serum significantly increased ($p < 0.001$) in alloxan treated animals when compared to control. SGPT levels of animals treated with MEBF 100 mg/kg p.o. & 200 mg/kg p.o. and glibenclamide (10 mg/kg p.o.) showed significant decrease ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 7

Effect of MEBF on Superoxide Dismutase (SOD)

The superoxide dismutase (SOD) level in pancreas significantly decreased ($p < 0.001$) in alloxan treated animals when compared to control. SOD levels of animals treated with MEBF 100mg/kg p.o. & 200 mg/kg p.o. and glibenclamide (10 mg/kg p.o.) showed significant increase ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 8.

Effect of MEBF on Catalase (CAT)

The catalase (CAT) level in pancreas significantly decreased ($p < 0.001$) in alloxan treated animals when compared to control. Catalase levels of animals treated with MEBF 100 mg/kg p.o. & 200 mg/kg p.o. and glibenclamide (10 mg/kg p.o.) showed significant increase ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 9.

Effect of MEBF on Glutathione peroxidase (GPx)

The glutathione peroxidase (GPx) level in pancreas significantly decreased ($p < 0.001$) in alloxan treated animals when compared to control. Glutathione peroxidase levels of animals treated with MEBF 100 mg/kg p.o. & 200 mg/kg p.o. and glibenclamide (10 mg/kg p.o.) showed significant increase ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 10.

Effect of MEBF on Lipid peroxidation

The lipid peroxidation level in pancreas significantly increased ($p < 0.001$) in alloxan treated animals when compared to control. Lipid peroxidation levels of animals treated with MEBF 100 mg/kg p.o. & 200 mg/kg p.o. and glibenclamide (10 mg/kg p.o.) showed significant decrease ($p < 0.001$) when compared to alloxan treated animals. Results are shown in Table 11.

Histopathological studies

Histopathological studies of pancreas of control group showed normal acini ducts with β islet cells, while diabetic control showed reduction in β islets cells with dilated and atrophic islets. Groups treated with standard drug showed proliferative hyperplastic β - islets and groups treated with MEBF at 100mg/kg showed β cells with increase in islets size, while MEBF treated group at dose of 200mg/kg showed increase in islets size and acini with two large hyperplastic islets. Histopathological studies revealed treatment by methanolic extract of *Butea frondosa* reduced pancreatic damage caused by alloxan. Results are shown in figure- 1.

Table 2: Effect of MEBF on total protein.

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200mg/kg)
Total Protein in serum (mg/dl)	7.60 ± 0.14	6.21 ± 0.13 ^{a*}	6.96 ± 0.11 ^{b*}	6.95 ± 0.12 ^{b*}	7.08 ± 0.12 ^{b*}

Values are mean ± SEM of 6 animals in each group
 Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.
 Statistical difference are expressed as * p<0.001.

Table 3: Effect of MEBF on haemoglobin

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	Group V MEBF (200 mg/kg)
Haemoglobin in blood (g/100 ml)	15.03 ± 0.15	9.73 ± 0.16 ^{a**}	13.23 ± 0.29 ^{b**}	10.51 ± 0.10 ^{b*}	13.93 ± 0.23 ^{b**}

Values are mean ± SEM of 6 animals in each group
 Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.
 Statistical difference are expressed as * p<0.01; ** p<0.001

Table 4: Effect of MEBF on total cholesterol

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Total Cholesterol in Serum (mg/dl)	130.18 ± 2.70	187.75 ± 2.05 ^{a*}	148.53 ± 1.75 ^{b*}	175.53 ± 0.99 ^{b*}	163.45 ± 1.64 ^{b*}

Values are mean ± SEM of 6 animals in each group
 Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.
 Statistical difference are expressed as * p<0.001

Table 5: Effect of MEBF on HDL Cholesterol

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
HDL Cholesterol in serum (mg/dl)	37.76 ± 1.02	29.48 ± 0.82 ^{a**}	43.55 ± 0.54 ^{b**}	32.39 ± 0.089 ^{b*}	37.65 ± 0.86 ^{b**}

Values are mean ± SEM of 6 animals in each group
 Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.
 Statistical difference are expressed as * p<0.05; ** p<0.001

Table 6: Effect of MEBF on Serum Glutamate Oxaloacetate Transaminase (SGOT)

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Serum Glutamate Oxaloacetate Transaminase (units/ml)	20.66 ± 1.52	56.16 ± 1.58 ^{a*}	29.66 ± 1.22 ^{b*}	31.33 ± 0.99 ^{b*}	26.5 ± 2.58 ^{b*}

Values are mean ± SEM of 6 animals in each group
 Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.
 Statistical difference are expressed as * p<0.001.

Table 7: Effect of MEBF on Serum Glutamate Pyruvate Transaminase (SGPT)

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Serum Glutamate Pyruvate Transaminase (SGPT) (units/ml)	23.66 ± 0.95	64.16 ± 3.26 ^{a*}	30.83 ± 1.38 ^{b*}	33.5 ± 0.76 ^{b*}	26.34 ± 1.02 ^{b*}

Values are mean ± SEM of 6 animals in each group
 Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.
 Statistical difference are expressed as * p<0.001

Table 8: Effect of MEBF on Superoxide Dismutase

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Superoxide Dismutase in pancreas (Units/mg protein)	1.423±0.019	0.501 ± 0.015 ^{a*}	1.255 ± 0.017 ^{b*}	1.009 ± 0.001 ^{b*}	1.281 ± 0.006 ^{b*}

Values are mean ± SEM of 6 animals in each group

Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.

Statistical difference are expressed as * p<0.001

Table 9: Effect of MEBF on Catalase (CAT)

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Catalase (CAT) in pancreas (nmole of H ₂ O ₂ decomposed/min/mg of protein)	1.025±0.089	0.321 ± 0.023 ^{a*}	0.961 ± 0.03 ^{b*}	0.823 ± 0.078 ^{b*}	0.923 ± 0.094 ^{b*}

Values are mean ± SEM of 6 animals in each group

Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.

Statistical difference are expressed as * p<0.001

Table 10: Effect of MEBF on Glutathione peroxidase (GPx)

Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Glutathione peroxidase (GPX) in pancreas (n moles of glutathione oxidised/min/mg of protein)	2.607±0.006	0.858 ± 0.035 ^{a*}	2.076 ± 0.04 ^{b*}	1.928 ± 0.019 ^{b*}	2.483 ± 0.024 ^{b*}

Values are mean ± SEM of 6 animals in each group

Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.

Statistical difference are expressed as * p<0.001

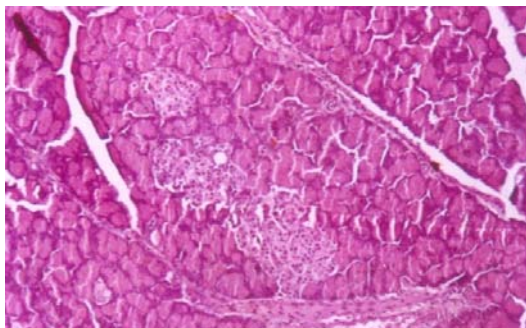
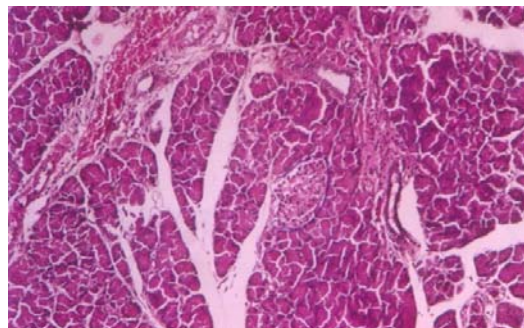
Table 11: Effect of MEBF on Lipid peroxidation

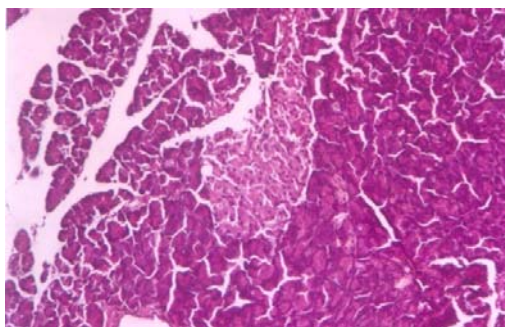
Group	Group I Control	Group II Diabetic Control	Group III Glibenclamide (10 mg/kg)	Group IV MEBF (100mg/kg)	GroupV MEBF (200 mg/kg)
Lipid peroxidation in pancreas (n mole of MDA formed/ min/mg of protein)	2.662 ± 0.34	8.056 ± 0.11 ^{a*}	3.053 ± 0.27 ^{b*}	4.101 ± 0.10 ^{b*}	3.245 ± 0.73 ^{b*}

Values are mean ± SEM of 6 animals in each group

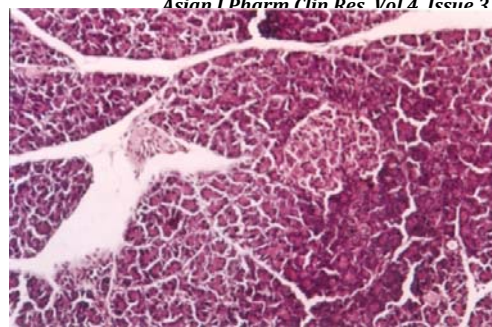
Comparison between: a - Group I and Group II, b - Group II and Group III, IV, V.

Statistical difference are expressed as * p<0.001

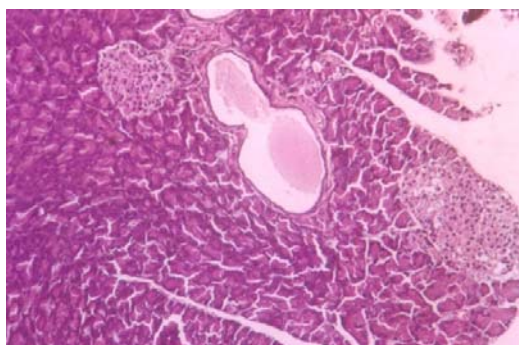
**Control animal (Group I)****Alloxan diabetic animal (Group II)**



Glibenclamide treated animal (Group III)



MEBF (100 mg/kg) treated animal (Group IV)



MEBF (200 mg/kg) treated animal (Group V)

Fig. 1: Histopathology of pancreas

DISCUSSION

Methanolic extract of *Butea frondosa* leaves at dose of 100 mg/kg and 200 mg/kg treated groups showed considerable improvement in all the parameters studied in a dose dependant manner and alloxan administration induced pronounced increase in the concentration of blood glucose. A significant hyperglycemia was attained within 48hrs after alloxan administration. During diabetes, the excess glucose present in the blood react with haemoglobin to form glycosylated haemoglobin, so the total haemoglobin level is lowered in alloxan diabetic rats ¹⁷.

Administration of MEBF reversed the total haemoglobin level in alloxan diabetic rats to near normal. We have also noticed elevated serum lipids in alloxan diabetic rats. Lipids play an important role in the pathogenesis of diabetes mellitus and the level of serum lipids is usually raised in diabetes and such an elevation represents a risk factors for coronary heart disease. Decreased total cholesterol level and improved HDL level was seen with the methanolic extract of *Butea frondosa* treated groups.

In diabetic animal, change in level of serum enzymes are directly related to change in the metabolism in which the enzyme is involved. Hence, the improvement noticed in the levels of enzymes studied viz., SGOT, SGPT are as a consequence of improvement in the carbohydrate and fat and protein metabolism due to methanolic extract of *Butea frondosa*. The restoration of SGOT and SGPT to the normal level after extract treatment also indicates revival of insulin secretion to a normal level. SGOT and SGPT levels also act as indicator of liver function, hence restoration of normal levels indicate normal functioning of liver.

Alloxan induces diabetes by damaging the insulin secreting cells of pancreas leading to hyperglycaemia¹⁸. and an attempt was made to study histopathology of pancreas. Damage of pancreas in alloxan treated diabetic control rats and regeneration of β -cells by glibenclamide and extract under investigation was observed. Photomicrographical data in our studies reinforce healing of pancreas, by MEBF, as a possible mechanism of their antidiabetic activity. Hyperglycemia can increase oxidative stress and is acknowledged as pathogenic mechanism in diabetic complications ¹⁹.

Oxidative stress induced by alloxan has been shown to damage pancreatic β -cells and produce hyperglycemia in rats. Oxidative stress produced by alloxan was found significantly lowered by administration of methanolic extract of *Butea frondosa*, this was evident from significant decrease in the lipid peroxidation in pancreas. Superoxide dismutase, Catalase, glutathione peroxidase constitutes a mutually supportive team of defense against reactive oxygen species.

Superoxide dismutase is a metalloprotein and is a first enzyme involved in the antioxidant defense by lowering the steady state of oxygen radical. In hyperglycemia, glucose undergoes autoxidation and produce superoxides and it produce free radical then in turn leads to lipid peroxidation in lipoprotein. Catalase is a heme protein, localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of hydrogen peroxide to water and oxygen and thus protect the cell from oxidative damage produced by hydrogen peroxide.

Glutathione peroxidase catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of hydroperoxides ²⁰. Improvement in the levels of antioxidant enzyme after treatment with methanolic extract treated rats indicates oxidative stress elicited by alloxan had been decreased due to the effect of the extract.

The overexpression of antioxidant enzymes in diabetic rats treated with *Butea frondosa* methanolic extract implies that the antioxidant defense may be reactivated by active principle such as flavanoids present in *Butea frondosa* extract, with their resulting increase in the capacity of detoxification through enhanced scavenging of oxy radicals. Hence the probable mechanism of action of producing antihyperglycaemic action may be due to antioxidant potential of extract which is revealed by improvement in the levels of antioxidant enzymes in pancreas of alloxan diabetic rats and validate its claim in Indian system of medicine .

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