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Antioxidant potential of ethanolic extract of *Bauhinia tomentosa* (Linn) flower

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ABSTRACT

To combat oxidative stress due to diabetic, administration of moderate quality of ethanol extracts of the *Bauhinia tomentosa* (Linn.) flower were suggested. The objective of the present study was to assess the antioxidant efficacy of ethanol extracts of the *Bauhinia tomentosa* (Linn.) flower. In diabetic rats, oxidative damage was studied by assessing parameters such as thiobarbituric acid reactive substances (TBARS), Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione-S-transferase (GST) in liver and kidneys, and also serum glutamate oxaloacetate transferase (SGOT), serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), Total Protein in serum/liver/kidneys. The effect of co-administration of ethanol extracts of the *Bauhinia tomentosa* (Linn.) flower on the above parameters was further investigated. Lipid peroxidation as evidenced by an increment in the values of TBARS, Hydroperoxides and also a distinct diminution of levels of GSH in diabetic rats was found to be near normalcy. Antioxidant enzymes such as SOD, CAT, GPx and GST too showed enhanced activities on co-administration of ethanol extracts of the *Bauhinia tomentosa* (Linn.) flower.

Keywords: *Bauhinia tomentosa*, anti-oxidant, TBARS, SOD, CAT

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INTRODUCTION

In response to the increased popularity and greater demand for medicinal plant, a number of conservation groups are recommending that wild medicinal plant be brought into cultivation.

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. Recently some synthetic antioxidants such as butylated hydroxyl toluens (BHT) and butylated hydroxyl anisole (BHA) have been suspected to dangerous to human health. Therefore, there is an urgent need to search for novel antioxidants from natural sources, which could be used in medicine and additive to nutriceuticals. The plant often contain substantial amount of antioxidants, such as flavonoids, carotenoids and tannins, flavones, isoflavones, anthocyanins, coumarin lignans, catechins and isocatechins[1]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like Diabetes, Atherosclerosis, Stoke, Alzheimer's disease and Cancer [2].

Thus an attempt was made to investigate the effect of *Bauhinia tomentosa* (Linn.) flower on antioxidant enzymes in liver of streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The flower of *Bauhinia tomentosa* (Linn.) were collected from an open field around B.Komarapalayam, Namakkal district Tamilnadu. Mr.G.V.S Murthy carried out identification of the plant at the Botanical Survey of India, Coimbatore-641003.

Preparation of plant extract

The flowers of *Bauhinia tomentosa (Linn.)* were first washed several times with distilled water and dried well. The flowers were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with petroleum ether, chloroform, acetone, ethanol, and aqueous using cold percolation method. The percentage yields were 1.54% in petroleum ether, 0.53% in chloroform, 2.1% in acetone, 12.3% in ethanol, and 6.0% in aqueous.

Preliminary phytochemical screening

One gram of the petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of *Bauhinia tomentosa (Linn.)* flowers were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening following the methodology of Harborne (1998) [3] and Kokate (2001) [4]. The result obtained in the present investigation (Table 1) petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of the flower of *Bauhinia tomentosa (Linn.)* showed the presence of carbohydrates, glycosides, alkaloids, phytosteroids, flavonoids, saponins, tannins & phenolic compounds and fixed oils & fats. Further, extracts of the flowers' showed the absence of proteins & amino acids and gums & mucilage.

Experimental work

The study was conducted on forty matured Wistar strain male albino rats; 3 months of age weighing about 150 ± 10 g. Animals were acclimated for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages (six rats per cage), at an ambient temperature of 25 ± 2^0C with 12 h light: 12 h dark cycle. Rats have free access to standard food and water *ad libitum*. The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout the duration of experiment and instruction given by our

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institutional ethical committee was followed regarding injection and other treatment of the experiment. Normoglycemic animals were selected for this experiment having the fasting blood glucose level of 75±5 mg/dl.

Experimental design

Diabetes was induced in rats within 48 hours by the intra peritoneal administration of streptozotocin dissolved in distilled water (5%) in a dose of 100 mg/kg body weight. The rats were divided into 5 groups (6 animals / Batch).

Group I: Control received Normal saline only (10 ml/Kg).
Groups II: Diabetic control, received streptozotocin and saline.
Groups III and IV: received streptozotocin and 48 hours later they were treated orally with hydro-ethanolic extracts of *Bauhinia tomentosa* at doses of 100 and 200 mg/kg.
Group V: Was treated with glibenclimide (600 µg/kg) as standard.

All the group of animals received the treatment by the above schedule for 15 days. Blood samples were collected one hour after drug administration and 7^{th} day to determine the blood glucose level.

Changes in liver profile

The serum liver profile values of SGOT, SGPT, ALP, and Total protein of those treated with EEBT returned to values nearer to control group. This showed that treatment with EEBT significantly improved the liver profile in diabetic animals showed in table no 1.

Changes in antioxidant enzymes

The concentration of tissues SOD, CAT, GST, GSH and GPx were significantly decreased in diabetic rats when compared to the control group. Administration of EEBT and glibenclamide to diabetic rats tend to bring the activities of these enzymes to near normal level showed in tables no 2.

The concentration lipid peroxidation and hydroperoxides in the liver and kidneys of both control and experimental groups of rats. There was a significant elevation in tissue lipid peroxidation and hydroperoxides in diabetic rats. Administration of EEBT or glibenclamide to diabetic rats decreased the levels of tissue lipid peroxidation and hydroperoxides to normal levels showed in tables no 3.

RESULTS

TBARS and Hydroperoxides contents registered an increase, where as GSH showed a distinct diminution in the liver and kidneys of groups II animals, where compared with group I (Table: 3), the values of the parameters returned towards near normalacy in group in rats.

Activity of antioxidant enzymes in different groups are given in Table 2. All these enzymes viz., SOD, CAT, GST, GSH and GPx showed a marked elevation in activity in liver and kidneys of diabetic group II rats. The activities of the enzymes were found to increase further significantly in group III and IV rats co-administered with EEBT.

Table 1 depicts the data relating to the levels of SGOT, SGPT, ALP, and Total protein in serum/liver/kidneys of different groups. All parameters showed a significant like in diabetic (group). They showed tendency to retrieve towards near normal values in EEBT administered group III and IV rats.

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Table No.1: Effect of EEBT and Glibenclamide in SGOT, SGPT, APL, Total protein of control and experimental groups of rats

Treatment	SGOT IU/L	SGPT IU/L	ALP U/L	Total protein (gm/dL)
Control	21.16 ± 04.30	18.7 ± 02.62	106.9 ± 02.86	6.8 ± 1.10
Diabetic Control	50.5 ± 06.50*	54.5 ± 04.40*	223.5 ± 07.9*	4.85 ± 0.22*
Diabetic + EEBT (100 mg/kg)	29.7 ± 04.04*	31.3 ± 01.90 *	138.4 ± 12.20*	5.2 ± 0.80*
Diabetic + EEBT (200 mg/kg)	39.0 ± 04.34*	40.6 ± 02.20*	147.7 ± 12.59*	6.46 ± 1.00*
Diabetic + Glibenclamide (600µg/kg)	25.0 ± 06.44*	26.6 ±0 5.12*	137.25±11.59*	7.75 ± 0.66*

SGOT - Serum glutamate oxaloacetate transferase, SGPT - Serum glutamate pyruvate transaminase, ALP – Alkaline phosphatase. Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *p<0.05.

Table No.2: Effect of EEBT and Glibenclamide in SOD, CAT, GPx, GST, GSH of control and experimental groups of rats

Groups	Control	Diabetic control	Diabetic + EEBT (100 mg/kg)	Diabetic + EEBT (200 mg/kg)	Diabetic + Glibenclamide (600µg/kg)
Superoxide dismutase (SOD)					
Liver	6.79 ± 0.27	4.10 ± 0.13*	4.51 ± 0.15*	5.01 ± 0.17*	5.84 ± 0.26*
Kidney	12.54 ± 0.72	6.82 ± 0.39*	8.98 ± 0.27*	9.48 ± 0.33*	10.43 ± 0.57*
Catalase (CAT)					
Liver	76.72 ± 2.26	55.14 ± 1.69*	72.05 ± 1.89*	74.65 ± 1.92*	69.57 ± 2.03*
Kidney	38.47 ± 1.57	29.29 ± 1.20*	42.06 ± 1.09*	43.87 ± 1.27*	35.92 ± 1.98*
Glutathione peroxidase (GPx)					
Liver	9.03 ± 0.47	5.29 ± 0.20*	7.16 ± 0.29*	7.77 ± 0.35*	7.68 ± 0.28*
Kidney	6.59 ± 0.22	4.41 ± 0.11*	4.51 ± 0.12*	4.76 ± 0.16*	6.34 ± 0.35*
Glutathione-S-transferase (GST)					
Liver	6.62 ± 0.33	3.80 ± 0.19*	4.02 ± 0.21*	5.21 ± 0.28*	6.02 ± 0.20*
Kidney	7.42 ± 0.24	2.10 ± 0.11*	2.80 ± 0.19*	3.01 ± 0.21*	3.74 ± 0.16*
Glutathione (GSH)					
Liver	40.19 ± 2.07	18.87 ± 0.98*	28.09 ± 1.89*	37.57 ± 2.31*	46.74 ± 2.80*
Kidney	24.20 ± 1.25	16.08 ± 1.25*	19.98 ± 1.42*	28.17 ± 1.60*	27.00 ± 1.61*

SOD - Superoxide dismutase, CAT – Catalase, GPx - Glutathione peroxidase, GST - Reduced glutathione, GSH - Glutathione-S-transferase, Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *p<0.05.

Table No.3: Effect of EEBT and Glibenclamide in LPO (TBARS AND HP) of control and experimental groups of rats

Groups	Control	Diabetic control	Diabetic + EEBT (100 mg/kg)	Diabetic + EEBT (200 mg/kg)	Diabetic + Glibenclamide (600µg/kg)
TBARS					
Liver	0.71 ± 0.03	1.67 ± 0.09*	0.80 ± 0.03*	0.88 ± 0.03*	0.98 ± 0.03*
Kidney	1.07 ± 0.04	1.92 ± 0.09*	0.81 ± 0.05*	0.98 ± 0.06*	1.20 ± 0.06*
Hydroperoxides					
Liver	78.74 ± 3.59	112.41 ± 4.50*	79.70 ± 4.02*	83.61 ± 4.02*	92.67 ± 4.58*
Kidney	48.54 ± 2.23	70.21 ± 3.27*	53.10 ± 2.25*	59.89 ± 2.25*	52.97 ± 2.24*

TBARS – Thio barbituric acid reactive substances, Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *p<0.05.

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DISCUSSION

Ample experimental and epidemiological studies support the involvement of oxidative stress in pathogenesis of many diseases. It is quite known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS can trigger a host of disorders in biological systems. Endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical induced damages of tissues. Oxidative stress is an outcome of imbalance between ROS production and antioxidant defaces, which in turn evokes a series of events deregulating the cellular function[5]. Elevated level of TBARS and Hydroperoxides in liver and kidneys of group II rats is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system. The significant decline in the concentration of these constituents in *Bauhinia tomentosa* administered rats unveils antioxidant efficacy of *Bauhinia tomentosa.* Decline in GSH content in the tissues of group II rats and its tendency to return to near normal in group III and IV animals too revealed the anti lipid per oxidative effect of *Bauhinia tomentosa.* The decrease in GSH content of liver in diabetic rats may probably be due to its increased utilization by the hepatocytes in an attempt to counteract the increased formation of lipid peroxides. Explanations of possible mechanisms underlying antioxidant potential of drug include the prevention of GSH depletion and destruction of free radicals. These two factors can be attributed to the antioxidant effect of *Bauhinia tomentosa.*

The antioxidant enzymes, such as SOD, CAT, GST and GPx constitute a mutually supportive team of deface against ROS [5]. They showed enhanced activities in EEBT rats as compare to controls. This may be due to the innate mechanism of the body to combat oxidative stress of a milder nature by secreting the enzymes in elevated levels. When the stress persists for a longer duration, and also turns to be severe, the inbuilt mechanism of the body falls to alleviate the damage. It is at this juncture that the EEBT is suggested to have triggered the secretion of antioxidant enzymes in enhanced levels which in turn stopped the oxidative damage due to diabetic. Further increment in the production of these in liver and kidney of EEBT co-administered animals may be due to the capacity of EEBT to stop the oxidative damage by hyperlipidemia. Similar observation was well documented [6].

GST plays an essential role in liver by eliminating toxic compounds by conjugating them with GSH. We found significant elevation in the activity of this enzyme in tissue of diabetic rats which registered a further significant like on EEBT co-administration. The initial elevation in the level of this enzyme may possibly be due to the self adjusting activity of the body to resist oxidative damage. The tendency of this enzyme to increase further EEBT co-administered animals clearly manifests the antioxidant activity of EEBT.

CONCLUSION

It is concluded that antidiabetic effect of *Bauhinia tomentosa* may be due to its ability to combat oxidative stress by quenching free radicals generated in the body as a result of Streptozotocin.

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