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ANTIOXIDANT ACTIVITY OF *ALBIZZIA LEBBECK* (LINN.) BENTH. IN ALLOXAN DIABETIC RATS

C. R. RESMI, M. R. VENUKUMAR* AND M. S. LATHA

Pharmacognosy Research Laboratory,
School of Biosciences,
Mahatma Gandhi University,
P.D. Hills P.O., Kottayam – 686 560

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Abstract : There is an increasing demand for natural anti-diabetic drugs, as continuous oral administration of insulin can culminate in many side effects and toxicity. In our endeavour to formulate some cost–effective herbal medicines for diabetes, we undertook this study to evaluate the antioxidant potential of aqueous extract of *Albizzia lebbeck* (ALL) in diabetic rats. The oxidative stress in alloxan-induced diabetic rats was determined by estimating the levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and reduced glutathione (GSH) in liver and kidneys. Activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione S transferase (GST) were assessed in diabetic as well as rats co-administered with ALL. Oxidative damage in the liver and kidneys of diabetic rats as evidenced by a marked increment in the levels of TBARS and CD, and also a distinct diminution in GSH content was nullified by ALL, as these parameters showed a tendency to retrieve towards normalcy on co-administration of the herbal drug. The antioxidant enzymes registered a decline in activity in diabetic rats thus revealing the damaging effects of free radicals generated due to alloxan exposure. The activities of these enzymes returned to normalcy in ALL-administered rats indicating the antioxidant efficacy of the drug in resisting oxidative insult. The findings provide a rationale for further studies on isolation of active principles and pharmacological evaluation.

Key words : *Albizzia lebbeck* alloxan antioxidant activity diabetes

INTRODUCTION

Diabetes mellitus is associated with oxidative stress (1), and hence an antidiabetic drug should possess antioxidant activity to resist such an oxidative insult of tissues. The free radical abuse in diabetes can be attributed to factors such as increased non-enzymic as well as oxidative glycosylation, metabolic stress due to changes in energy metabolism, the levels of intermediary mediators and also the status of antioxidant defense systems. (2) Antioxidant potential of plants such *as Piper*

*Corresponding Author : P.G. & Research Department of Zoology, N.S.S. Hindu College, Changanassery – 686 102 (Kerala); E-mail : venukumarmr2000@yahoo.co.in

nigrum (3). *Syzygium cumini* (4), *Tinospora cordifolia* (5), *Momordica charantia* (6), *Azadirachta indica* (7), *Ocimum sanctum* (8), *Allium sativum* (9) etc. has been documented. In our laboratory, we have established antioxidant potential of plants like *Coscinium fenestratum* (10), *Curculigo orchioides* (11) and *Syzygium aromaticum* (12). The present study was designed to investigate the protective effect of *Albizzia lebbeck* leaves in lowering the blood glucose level, lipid peroxidation and antioxidant enzyme status in alloxan-induced diabetic rats.

Albizzia lebbeck (Linn.) Benth. of family Mimosaceae is a medium to large sized tree distributed throughout India. The plant is used against cataract, asthma, ophthalmopathy, leprosy, diarrhoea and all types of poisoning (13). Anti-allergic (14), anti-inflammatory (15) and anticonvulsant (16) activities of this plant have been documented.

MATERIAL AND METHODS

Plant material : The leaves *of A. lebbeck* were locally collected during June–August. The plant was identified and authenticated by the experts in the Post Graduate and Research Department of Botany, St. Thomas College, Pala, Kottayam District. The leaves were chopped, air dried for a week and powdered. The powder was then extracted in distilled water using Soxlet extractor. The extract was concentrated in a rotary evaporator under reduced pressure (yield: 1.48% w/w). The concentrate thus obtained (ALL) was stored in airtight polythene containers for further use.

Experimental animals : Male albino rats of Sprague-Dawley strain weighing 120–150 g were secured from Small Animals' Breeding Centre of Kerala Agricultural University, Mannuthy, Trichur. They were housed in polypropylene cages maintained at controlled room temperature and normal light-dark cycle. The animals were fed on pellet diet (supplied by Hindustan Lever, India) and water *ad libitum.* A week's time was given for the animals to get acclimatized with the laboratory conditions.

Experimental induction of diabetes : The rats were administered with alloxan monohydrate (supplied by S.D. Fine Chemicals Ltd., Boisar) dissolved in sterile normal saline at the dose of 150 mg/kg body weight, ip (intraperitoneally). Since alloxan could evoke fatal hypoglycemia as a result of massive insulin release, rats were treated with 15 ml 20% glucose solution ip, 6 h after alloxan treatment. The rats were then kept for next 24 h with free access to 5% glucose solution to prevent hypoglycemia. After a fortnight, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia (evidenced by blood glucose range of 200–260 mg/100 ml) were selected for the experiment (17).

Experimental design : Animals were divided into 3 groups of 6 rats each as follows. Group I animals consisted of normal rats supplied with pellet diet and water *ad libitum.* They also received saline at the dose of 10 ml/kg body weight, ip. Group II animals were the alloxan-diabetic rats supplied with pellet diet and water *ad libitum.* Group III animals constituted alloxan–diabetic rats co-administered ALL at the dose of 75 mg/kg

body weight, twice a week, po (orally) in 2 ml of distilled water for a period of four weeks. (A pilot study revealed that ALL caused anti-diabetic effect at doses ranging 25–125 mg/kg body weight. 75 mg was found to be the effective dose)

The animals were reared in laboratory conditions for a period of four weeks. After that, the animals were fasted overnight and then sacrificed under light anaesthesia (ether inhalation). Blood was collected from jugular vein in two separate tubes. Blood collected in the tube containing potassium oxalate and sodium fluoride was used for estimating blood glucose. The blood contained in the second tube was allowed to clot at room temperature and serum separated after centrifugation.

Tissues like liver and kidneys were dissected out, blotted off blood, rinsed in ice-cold saline and weighed. Fat was freed from the tissues (18) and then homogenized in buffer containing 50 mM Mannitol, 2 mM Tris HCl pH 7 (10%) in a Potter Elvehjem homogenizer fitted with a polyteflon plunger at high speed. The homogenate thus obtained was centrifuged at 25000 rpm at 4°C. The supernatant fraction was used for various biochemical estimations.

Biochemical estimations: Estimations of TBARS (19), CD (20) and GSH (21) were made in liver and kidneys of different experimental groups. Activities of antioxidant enzymes, such as SOD (22), CAT (23), GPX (24) and GST (25) were assayed in different tissues. Blood glucose level (26), liver glycogen content (27) and protein content of tissue homogenates (28) were also measured.

Statistical analysis

The results were presented as the mean±SEM. Student's paired t test was used to analyze statistical significance.

RESULTS

The extent of diabetes observed in different experimental groups is given in Table I. A significant increase in blood glucose level, and a marked depletion in liver glycogen content were observed in alloxan-induced diabetic rats. The blood glucose level and hepatic glycogen content attained normalcy with ALL co-administration.

TABLE I: Effect of *A. lebbeck* on contents of blood glucose and liver glycogen.

Parameters	*Group-I*	*Group-II*	*Group-III*
Blood glucose (mg/100 ml)	110.4±2.1	297.8±3.9*	133.2±2.8^
Liver glycogen (mg/100 g)	1093±2.6	807±2.8*	1064±2.3^

Values are mean±SEM of 6 animals in each group.
*P<0.01 as compared to Group-I.
^P<0.01 as compared to Group-II.

Table II summarizes the effect of co-administration of ALL to alloxan-induced diabetic rats on TBA-reactive substances, conjugated dienes and glutathione levels. There was a significant increase in TBARS and CD levels while GSH levels in liver and kidney showed marked depletion in Group II. Administration of ALL lowered these high levels of TBARS and CD while GSH levels were enhanced to normalcy in Group III rats.

TABLE II: Effect of *A. lebbeck* on antioxidant status of liver and kidneys.

Parameters		*Group-I*	*Group-II*	*Group-III*
TBARS (µmol/100 g tissue)	In liver	0.7±0.02	1.1±0.03*	0.8±0.02^
	In kidney	1.0±0.02	1.4±0.04*	1.0±0.03^
CD (µmol/100 g tissue)	In liver	0.3±0.03	0.7±0.05*	0.4±0.05^
	In kidney	0.5±0.02	0.9±0.03*	0.5±0.02^
GSH (µmol/100 g tissue)	In liver	482.1±2.5	404.6±4.2*	499.2±3.5^
	In kidney	380.6±2.1	319.6±1.6*	371.7±1.9^

Values are mean±SEM of 6 animals in each group.
*P<0.01 as compared to Group-I.
^P<0.01 as compared to Group-II.

Activities of antioxidant enzymes are presented in Table III. SOD, CAT, GPX and GST activities showed marked reduction in alloxan-treated rats when compared with the normal controls. On ALL co-administration, the activities of these enzymes attained normalcy.

TABLE III: Effect of *A. lebbeck* on antioxidant status of liver and kidneys.

Parameters		*Group-I*	*Group-II*	*Group-III*
SOD (units/mg protein)	In liver	6.4±0.16	2.8±0.16*	6.0±0.15^
	In kidney	6.0±0.15	1.9±0.14*	5.7±0.09^
CAT (µmol of H_2O_2/min/mg protein)	In liver	211.7±5.27	135.3±2.07*	202.8±1.09^
	In kidney	73.1±0.8	31.8±1.1*	71.6±0.9^
GPX (units/mg protein)	In liver	166.4±1.5	97.7±1.4*	155.3±2.3^
	In kidney	123.1±2.1	59.6±1.5*	116.6±1.9^
GST (units/mg protein)	In liver	0.87±0.06	0.25±0.03*	0.79±0.4
	In kidney	0.80±0.07	0.41±0.01*	0.73±0.02^

Values are mean±SEM of 6 animals in each group.
*P<0.01 as compared to Group-I.
^P<0.01 as compared to Group-II.

DISCUSSION

Free radicals mediated tissue damage occurs in the generation and progression of diabetes mellitus. Insulin secretion is impaired during diabetes and this may evoke lipid peroxidation in biological systems (1).

Enhanced levels of TBARS and CD observed in the tissues of diabetic rats indicate excessive formation of free radicals and activation of lipid peroxidative system. The tendency of these parameters to retrieve towards near normal values in ALL co-administered rats unveils the anti–lipid peroxidative potential *of A. lebbeck*. This finding matches with that of other investigators (2, 29).

Reduced glutathione (GSH) is essential to maintain structural and functional integrity of cells. Apart from its direct free radical scavenging properties and abilities to conjugate with several electrophilic intermediates that are capable of initiating lipid peroxidation, GSH acts as the physiological co-substrate of the conjugating enzyme system. The distinct diminution in GSH content of tissues in diabetic rats and its subsequent attainment of near normalcy on ALL administration reveal the protection offered by *A. lebbeck* in combating oxidative insult due to diabetes. This observation is in agreement with the findings of Prince et al (2).

Decline in the activities of antioxidant enzymes, such as SOD, CAT, GPX, GST etc. observed in diabetic rats indicate the extent of free radical induced damage due to hyperglycemia. It is now known that, when there is an imbalance between free radical

production and antioxidant defenses, 'oxidative stress' occurs resulting in deregulation of cellular functions (30). An antioxidant drug is expected to bring an alleviation of this type of cellular dysfunctions. The profound increment in the activities of the antioxidant enzymes in ALL-co-administered rats unravels the efficacy of the drug in resisting oxidative insult due to diabetes.

The enhanced levels of blood glucose as well as decline in liver glycogen content in diabetic rats, and its return towards near normal values in ALL-administered animals manifest the hyperglycemic activity of alloxan as well as hypoglycemic effect of ALL.

It can be concluded that *Albizzia lebbeck* seems to be a promising plant in respect to its antioxidant potential to alleviate diabetes, and it necessitates further studies.

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