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BIOCHEMICAL EVALUATION OF HYPOGLYCEMIC ANTIOXIDANT POTENTIAL OF HERBAL EXTRACT STUDIED IN (STZ) STREPTOZOTOCIN INDUCED DIABETIC RAT

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ABSTRACT

Diabetes Mellitus is a clinical condition characterized by increased blood glucose level due to the insufficient insulin. Streptozotocin is to induce hyperglycemic condition. Luffa acutancula and Gmelina arborea may be the good remedy for the treatment of diabetes mellitus. In this study, the oral administration of Luffa acutancula and Gmelina arborea showed the hypoglycemic activity and it could exert a beneficial action against biochemical alterations caused by the streptozotocin. Diabetes mellitus is a disease that affects more than 100 million people and may attain about five times more subjects in the next 10 years. In the search for new compounds, and within the exploratioration of natural resources, the hypoglycemic effect of plants which are reputed antidiabetic. The present study is carried out to identify new potential antidiabetic compounds of Luffa acutancula and Gmelina arborea. In the present study the antioxidative potential of Luffa acutancula and Gmelina arborea was assessed in streptozotocin induced diabetic rats. Lipid peroxide levels were also measured in normal, diabetic and treated animals. Malondialdehyde (MDA) levels were significantly higher and antioxidant activity was found low in diabetic groups as compared to the control groups, and significant alteration in both the MDA levels and antioxidant activity was also observed when the above herbal hypoglycemic agents were given to diabetic rats. On the basis of our results we conclude that Luffa acutancula and Gmelina arborea are not only useful in controlling the lipid peroxide levels but are also helpful in further strengthening the antioxidant potential. The probable mechanism by which Luffa acutancula and Gmelina arborea exerts its protective action against streptozotocin-induced pancreatic metabolic alterations could be by the stimulation of pancreatic regeneration through an improved synthesis of protein or accelerated detoxification and exertion. Furthermore, comprehensive chemical and pharmacological research is required to reveal the mechanism of the anti-diabetic potential of Luffa acutancula and Gmelina arborea.

KEY WORDS

Free radicals, Diabetes mellitus, Antioxidants, Luffa acutancula and Gmelina arborea, Streptozotocin (STZ).

INTRODUCTION

Our Country has immense wealth of huge medicinal plants. These plants have credited to the development of therapeutic agents for various ailments and diseases. The medicinal plants documented in 'Rig Veda', (4500-1600 B.C) mentions 67 medicinal plants. 'Yajur Veda' 81 plants and 'Atharvana Veda', (4500-2500 B.C)

290 species, which are still used in classified formulations in Ayurvedic system of medicine. Diabetes Mellitus is the first leading cause of death (after heart disease and cancer) in many developed countries. It affects about 2% to 3% of the general population complications of diabetes affects the eye, kidney and nervous system. Diabetes is a major cause of blindness, renal failure, amputation, heart attack and strokes. It is a clinical condition characterized by insulin is either not produced in sufficient quantity or insufficient in its action on the target tissues.

Moreover, diabetes also induces changes in the tissue content and activity of the antioxidant enzymes Wohaieb S.A. *et al.,* (1987), Asayama K. *et al.,* (1989).Since the time of Charaka and Susruta many herbal medicines in different oral formulations have been recommended for Madhumeha and confident claims of cure are on record, Aslam M. *et al.,* (1998).

WHO Expert Committees, technical report series of Diabetes mellitus 1985,727. Diabetes mellitus remains a major health problem and prevention of diabetes still lies in the realm of future and until then tens of millions will continue to suffer from this disease. Oxidative stress is reported to be increased in patients with diabetes mellitus, Baynes J.W (1991). Role of oxidative stress in development of complications in diabetes. Diabetes 40, 405-412. Accumulating evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus Bambolkar S *et al.,* (1995). Reactive oxygen species generated in the cells are scavenged by antioxidant enzymes Genet S. *et al*., (2002).

It is well known that the plants like Luffa *acutancula* and Gmelina *arborea* are not only possess hypoglycemic activity but some of them are hypotensive, hepatoprotective and also blood purifier Tiwari A.K *et al.,* (2002), Grover J.K. *et al.,* (2002).

In view of the above considerations the present study was designed to investigate the protective effect of Luffa *acutancula* and Gmelina *arborea* on plasma lipid peroxide levels and on anti-oxidant enzyme superoxide dismutase (SOD). Moreover, antioxidant molecules, uric acid and albumin content were also measured in streptozotocin induced diabetic rats.

SCOPE OF OUR STUDY

In this study the use of plants for medicinal purpose used locally in the treatment of various diseases and we examined for their antioxidant activity.

The results of our studies conducted in animals have reported that useful in controlling the lipid peroxide levels but are also helpful in further strengthening the antioxidant potential. Therefore, the present investigation is part of continuing programme related to the biochemical screening of local plants used in Ancient Indian Medicine, Ayurveda, Siddha and Yunani. In several countries including India several plant species are administered orally to control the diabetes mellitus. Some of these plants have been pharmacologically provided to be of some value in diabetes mellitus. Luffa *acutancula* and Gmelina *arborea* may have the popular remedy for the treatment of diabetes mellitus.

MATERIALS AND METHODS

CHEMICALS

All the fine chemicals were purchased from Sigma chemical co., USA. All other chemicals used were of good quality and analytical grade.

Luffa *acutancula* and Gmelina *arborea* as a gift from the Siddha Maruthuva Salai Vellore, Tamilnadu. All the plants were identified taxonomically by Dr.N.P.M.Mohammed Tariq (Botanist) Department of Biotechnology, Islamiah College (Autonomous) Vaniyambadi.

PREPARATION OF PLANT EXTRACT

Luffa *acutancula*: Air dried plant leaves (100 g) were boiled in 200 ml of distilled water for 10minutes. After cooling to room temperature, the supernatant was filtered to obtain the decoction ready for animal treatment Satyanarayan K. *et al.,*(1978).

Gmelina *arborea*: Air dried leaves powder was boiled in distilled water for 10minutes. After cooling to room temperature, the supernatant was filtered to obtain the decoction ready for animal treatment Luthy N. *et al.,* (1964).

ANIMALS

Male albino rats weighing 150-200 g were used in the present study. All rats were kept at room temperature of 20°C in the animal room of our Department of Biochemistry, Islamiah College (Autonomous) Vaniyambadi. They were maintained on food pellets and water *ad libitum.* 18 rats, included for the study, were divided into 6 groups, each consisting of three animals. Out of 6 groups, seven were made diabetic with a single dose of streptozotocin (65 mg/kg b.w.) by intraperitoneal route Shibib B.A. *et al.,* (1993).

Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day post administration of streptozotocin. Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Rats were divided into the following groups.

SEGGREGATION OF EXPERIMENTAL GROUPS

Group 1: Control given only saline (10 ml/kg/once a day, daily)

Group 2: Streptozotocin induced diabetic given in saline (10 ml/kg/once a day, daily)

Group 3: Diabetic rats treated with Luffa *acutancula (*10 ml/kg/ once in a day, daily)

Group 4: Diabetic rats treated with Gmelina *arborea (*10 ml/kg/ once in a day, daily)

Group 5: Diabetic rats treated with Insulin (5 units/ kg/ once a day, daily)

Group 6: Diabetic rats treated with Glibenclamide (500 mg/kg/ once a day, daily)

After 30 days of treatment the body weight and fasting blood glucose of the animals were again determined. Blood was collected in heparinized vial and in plain vial for hemolysate preparation and for serum separation respectively.

PREPARATION OF HEMOLYSATE

The Collected blood was centrifuged for 10minutes at 3000 rpm. The plasma thus obtained was used for lipid peroxide estimation Ohkawa H. *et al.,* (1979). Remaining packed RBCs were washed thrice with normal saline to remove the buffy coat. Hemolysis was performed by pipetting out 1 ml of washed red blood suspension in ice cold double distilled water. Erythrocyte ghosts were sedimented in a high speed refrigerated centrifuge at 12000 rpm for 40 minutes. The cell content was separated out carefully and used for superoxide dismutase estimation McCord J.M. *et al.,* (1969).

ISOLATION OF ERYTHROCYTES AND ERYTHROCYTE MEMBRANES

Erythrocytes and their membranes were isolated from the control and experimental groups according to the method of Dodge *et al.*, with slight modifications. Packed cells were washed with isotonic saline to remove buffy coat. Different aliquots of packed cells were thoroughly washed with Tris-buffer 0.31 M (pH7.4). These were used for the assay of various biochemical parameters. Then, another aliquot of packed cells were subjected to hemolysis by adding hypotonic Tris-buffer 0.015 M (pH7.2). After 4-6 hrs, the erythrocyte ghosts were sedimented by centrifugation at 12,000 rpm for 40-45 at 4^{o} C, the supernatant (hemolysate) was used for the analysis of antioxidants. The erythrocyte membrane pellets were suspended in 0.02 M Tris-buffer (pH 7.2) and used for various other biochemical estimations.

SEPARATION OF SERUM

The blood collected in plain vial was kept for some time. Serum from blood after clotting separated out and collected in clean dry centrifuge tube and again centrifuged for 5minutes at 3000 rpm. The serum thus obtained was used for albumin and uric acid estimations Rodkey F.L. *et al.,* (1965), Eichhorn E. *et al.,* (1961).

RESULTS AND DISCUSSIONS

In streptozotocin induced diabetic rats there was a significant ($p<0.001$) increase in fasting blood glucose (133.34%) and a comparative decrease ($p<0.001$) in body weight and protein content. There was a slight increase in body weight and protein and a significant decrease in fasting blood glucose (**Table 1**) in diabetic rats treated with Luffa *acutancula* (55.13), Gmelina *arborea* (71.21%). These effects are quite similar to that obtained by insulin and glibenclamide. **Table 2** shows a statistically significant increase in lipid peroxide levels ($p<0.001$) in streptozotocin induced diabetic rats with respect to normal controls and there was a significant decrease in lipid peroxide levels in diabetic rats treated with herbal preparations of Luffa *acutancula* ($p<0.001$), Gmelina *arborea* ($p<0.001$). In contrast to this, the activity of the enzyme superoxide dismutase (SOD) was significantly decreased in diabetic rats as compared to that of normal control (**Table 2**). All the anti-diabetic agents including herbal preparations, insulin and glibenclamide used in the present study significantly elevated the activity of superoxide dismutase when compared with diabetic control. Another antioxidant, albumin was also found decreased in diabetic group as compared to normal control (**Table 3**).

Following treatment with herbal preparations both showed some, significantly increased levels of albumin were found. Insulin and glibenclamide also showed significant ($p<0.001$) increase in albumin levels as compared to the control group (**Table 3**). Following treatment with both the herbal preparations increment ($p<0.01$) in the uric acid levels was observed, while insulin and glibenclamide exhibited non significant changes (**Table 3**).

Table 1: The effect of herbal hypoglycemic agent on body weight, blood glucose and protein in normal and diabetic rats. Values are expressed as mean + S.E. (n=6). Student's t-test.

S.no	Groups	Change in body weight	Fasting Blood Glucose (mg/dl)		Protein value (mg/ml)
			Pre-treated	Post-treated	
1	Control	+35.0±5.12	84.83±6.75	86.06±8.32	63.48±6.86
2	Diabetic control	-20.5±6.2***	192.60±6.91	195±10.78***	42.6±7.20***
3	Diabetic rats + Luffa *acutancula*	+10.4±7.2*	192.28±7.77	109.25±10.58***	46.18±10.65*
4	Diabetic rats + Gmelina *arborea*	+8.5±5.05*	197.93±10.62	78.45±6.48***	44.66±11.52*
5	Diabetic rats + Insulin	+16.3±6.4**	197.46±6.79	81.42±8.03***	47.68±9.51**
6	Diabetic rats + Glibenclamide	+0.6±5.35**	198±6.77	109.92±10.15***	46.65±6.30**

p-values: *p<0.1, **p<0.01, *** p<0.001.

Table 2: Effect of herbal hypoglycemic agents on the levels of lipid peroxide and superoxide dismutase. Values are expressed as mean + S.E. (n=6). Student's t-test.

S.No.	Groups	MDA (nmol/ml)	SOD (U/mg protein)
1	Normal Control Groups	6.98±0.395	92.7±7.21
2	Diabetic control	10.81±0.586***	65.5±6.45***
3	Diabetic rats + Luffa *acutancula*	7.41±0.148***	86.0±6.05***
4	Diabetic rats + Gmelina *arborea*	7.38±0.059***	77.5±5.31***
5	Diabetic rats + Insulin	6.21±0.075***	133.8±6.41***
6	Diabetic rats + Glibenclamide	6.83±0.261***	107.3±9.36***

***p<0.1, ***p<0.001 vs diabetic control group.**

Table 3: Effect of herbal hypoglycemic agents on the levels of albumin and uric acid. Values are expressed as mean + S.E. (n=6). Student's t-test.

S.No.	Groups	Albumin (g/dl)	Uric acid (mg/dl)
1	Normal Control Groups	6.74±0.173	0.685±0.020
2	Diabetic control	6.13±0.1030	0.572±0.059***
3	Diabetic rats + Luffa *acutancula*	6.67±0.1545***	0.493±0.0085**
4	Diabetic rats + Gmelina *arborea*	6.29±0.1365**	0.667±0.0292**
5	Diabetic rats +Insulin	6.74±0.1176***	0.544±0.0642*
6	Diabetic rats + Glibenclamide	6.67±0.0583***	0.535±0.0070*

***p<0.1, **p<0.01 and ***p<0.001 Vs diabetic control group.**

CONCLUSION

To conclude, our study suggested that the herbal plants posses the hypoglycemic effect or antidiabetic effect and antioxidant activities, which might be helpful in preventing or slowing the progress of diabetes. Further in vivo studies and investigations on the isolation and identification of active components in the plants may lead to chemical entities with potential for clinical use in the prevention and treatment of diabetes and various diseases and to evaluate the levels of metal ions such as copper, zinc, magnesium, manganese and selenium, as altered metabolism of these metals have been reported to occur in both IDDM and NIDDM Walter R.M. *et al.,* (1991).

The results of our present study demonstrated that the elevated plasma lipid peroxide levels in streptozotocin-induced diabetic rats along with a significant decrease in the anti-oxidant enzyme, superoxide dismutase activity. Moreover, we also found reduced levels of serum albumin and uric acid in diabetic rats. Earlier there have been many reports documenting elevated serum lipid

peroxide levels and diminished antioxidant status in diabetic subjects Sato Y.*et al.,*(1979), Oberley L.W. *et al.,*(1988).

They also significantly reduce the plasma lipid peroxide levels in diabetic rats. Moreover, following treatment the activity of the anti-oxidant enzyme superoxide dismutase and serum albumin content was also found increased. However, the serum uric acid content was not found significantly altered. Uric acid is one of the most abundant chain breaking antioxidants present in human serum and its levels are largely determined by genetic factors, purine intake and renal function Situnayake R.D. *et al*.,(1991).

Moreover, excess accumulation of urate in serum and tissues induce gouty pathology, and is by no means beneficial from the medical point of view Asayama K. *et al.,* (1993). SOD and albumin form the primary defense against reactive oxygen metabolites Mahdi A.A. (2002). Such metabolites have been implicated in the damage brought about by ionizing radiation, as well as in the effects of several cytostatic compounds Marklund S.L. *et al*., (1982).

The decreased activity of antioxidant molecules along with elevated lipid peroxide levels in diabetic rats could probably be associated with oxidative stress and/or decreased antioxidant defense potential Mahdi A.A. *et al*., (1996).The reversal in their content following treatment may be due to decreased oxidative load. Elevated SOD activity and albumin levels in insulin treated group may probably be due to the anabolic role of this proteinous hormone. The herbal hypoglycemic agents may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds Gupta S.K. *et al.,*(2002) or by increasing the synthesis of anti-oxidant molecules.

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