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A STUDY OF HYPOGLYCEMIC AND ANTIOXIDANT ACTIVITY OF *AEGLE MARMELOS* IN ALLOXAN INDUCED DIABETIC RATS

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Abstract : The present study was performed to evaluate the hypoglycemic and antioxidant effect of aqueous extract of *Aegle marmelos* leaves (AML) on diabetic rats. Male albino rats were randomly divided into three groups : Group I : Control; Group II : Diabetic rats; and Group III : Diabetic rats administered AML. Glucose, urea and glutathione-S-transferase (GST) in plasma, glutathione (GSH) and malondialdehyde (MDA) levels in erythrocytes were estimated in all the groups at the end of four weeks. There was a decrease in blood glucose at the end of four weeks in group III animals compared with group II, however it did not reach the control levels. There was an increase in erythrocyte GSH and a decrease in MDA in group III as compared to group II. The plasma GST levels were raised in diabetic rats when compared to controls. In the group III animals, there was a decrease in GST as compared to group II. Owing to hypoglycemic and antioxidant properties, AML may be useful in the long-term management of diabetes.

Key words : aegle marmelos, hypoglycemic action, antioxidant activity, diabetic rats

INTRODUCTION

Aegle marmelos has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India (1), Bangladesh (2) and Sri Lanka (3). *Aegle marmelos* (Sanskrit: Bilva) is reported to have hypoglycemic activity by Seema et al (4). In diabetes mellitus, there is oxidative stress associated with release of free radicals (5). Such an oxidative stress is important in the development of many of the complications of diabetes mellitus such as retinopathy and nephropathy (6, 7). In the present study, we have evaluated the hypoglycemic and antioxidant effects of aqueous extract of *Aegle marmelos* leaves (AML) on alloxan induced diabetic rats. Plasma glucose levels were assayed to assess the hypoglycemic activity. Plasma glutathione-S-transferase (GST), erythrocyte malondialdehyde (MDA) and erythrocyte glutathione (GSH) were estimated to assess

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the antioxidant activity.

METHODS

Preparation of the aqueous extract

About 500 gms of the shade dried powdered leaves were dissolved in 5 liters of distilled water and boiled for 5 hours to get 50 gms of the paste. About 100 mg of the paste was dissolved in 0.5 ml water just prior to the administration.

Animals

Male albino rats 3–4 months old, weighing 160–200 gms (mean 180 gms) were randomly divided into three groups. The group I animals were injected with normal saline and served as controls. The group II and group III animals were injected with alloxan (80 mg/kg body weight into the tail vein) to induce diabetes. After 5 days of alloxan injection, the rats with blood glucose level >120 mg% were randomly divided into group II and group III. Thus, animals in Group I (n = 9) were treated as normal control, while animals in Group II (n = 8) were alloxan induced diabetic rats, and animals in continuous Group III (n = 9) were alloxan induced diabetic rats administered with aqueous extract of AML. All the animals were reared on normal lab chow diet (Hindustan Lever Ltd.) for four weeks. The group III animals were daily administered the extract of AML via intragastric tube at a dose of 500 mg/kg body weight.

The fasting plasma glucose levels were serially estimated in the group III animals at the end of first week, second week and fourth week. At the end of four weeks, blood was collected into EDTA coated tubes by retro orbital puncture. The plasma was separated by centrifugation and was used for the measurement of glucose, urea and GST levels. GSH and MDA levels were estimated in the packed cells after discarding the buffy layer. The plasma was frozen till the assays were performed on the next day. The erythrocyte GSH and MDA were estimated on the same day after washing the packed cells twice with phosphate buffered saline. Erythrocyte MDA was estimated by its reaction with thiobarbituric acid to yield a pink chromogen read at 532 nm (8). It was expressed as nmol/gm of hemoglobin. Dithiobis nitrobenzoic acid was used for the GSH assay (9). GSH was expressed as mg/gm of hemoglobin. Plasma glucose was estimated by the o-toluidine method (10) and plasma urea by the diacetyl monoxime method (11). Plasma GST was determined spectrophotometrically using 1-chloro-2, 4-dinitrobenzene (12). Results were expressed as µmoles/min/100 ml plasma.

Statistics

The obtained data were analysed using the unpaired *t*-test between the groups. The plasma glucose levels at the end of 1st, 2nd and 4th week in group III animals was analyzed by the paired *t*-test. Correlation analysis was performed between the parameters and significance of correlation was assessed by the Fisher's r to z test. Statistical analysis was performed using Statview version 4 software package.

RESULTS

The body weights of all the animals were serially recorded every week to check their

well being. The mean±SD levels of all the parameters in the groups are tabulated in Table I. There was a significant reduction in the mean blood glucose level in diabetic rats on AML when compared to untreated diabetic rats. However, the blood glucose level in treated diabetic rats did not approach the glucose levels of the control group. Also in group III, there was a significant reduction in the blood glucose level at the end of 2nd week when compared to 1st week (P = 0.02, Fig. 1). In the 4th week after administration of the extract, there was a further decrease in blood glucose level, however it was not statistically significant when compared to the blood glucose levels at the end of 2nd week (Fig. 1). The blood urea levels decreased on treatment with the extract in group III animals, however it did not return to normal control levels. There was a significant increase in the GSH levels and a decrease in MDA in erythrocytes of group III animals compared to controls. Also, the mean GST levels were higher in diabetic rats and they decreased on administration of AML.

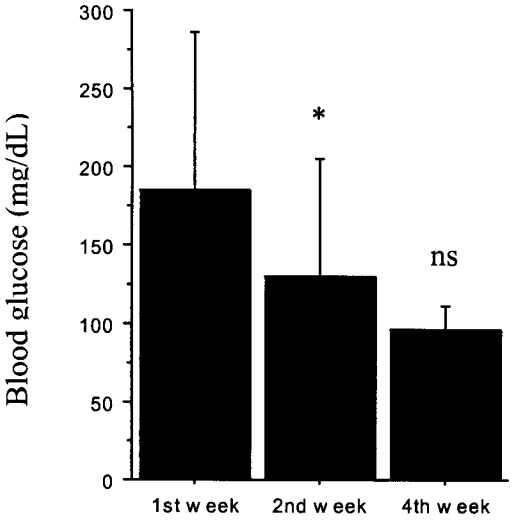

Fig. 1: Blood glucose levels at the end of 1st, 2nd and 4th weeks in the diabetic animals administered *Aegle marmelos* leaves (*P = 0.02 when compared to 1st week, ns- not significant when compared to 2nd week).

A significant positive correlation was observed between MDA and GST (r = 0.714, P<0.0001) and a negative correlation was observed between GSH and GST (r = –0.771, P<0.0001).

TABLE I: Blood glucose, urea, GSH, MDA and GST levels in control (group I) and diabetic rats without (group II) and with treatment (group III).

	Group I (Controls) n = 9	*Group II (Diabetic) n = 8*	*Group III (Diabetic with drug) n = 9*
Plasma Glucose (mg/dl)	58.333±10.000	156.875±49.637†	96.111±15.568⌘
Plasma Urea (mg/dl)	10.889±2.028	26.375±6.278†	17.111±4.076⊙
Erythrocyte MDA (nmol/gm Hb)	13.313±1.815	20.973±4.233◆	16.228±2.683□
Erythrocyte GSH (mg/gm Hb)	19.729±5.434	6.766±1.406†	14.861±4.946*
Plasma GST (µmol/min/dL)	10.993±2.511	18.420±2.046†	13.382±1.166†

Values are shown as means ± SD.
Group II was compared to Group I and Group III was compared to Group II.
†P<0.0001; ◆P=0.0002; ⌘P=0.003; ⊙P=0.002; *P=0.0005; □P=0.01

DISCUSSION

We have been able to demonstrate a significant hypoglycemic effect in the dose of 500 mg/kg body weight once daily. Similar hypoglycemic effects in rats was also observed by Karunanayake et al (3). There was an improvement in glucose tolerance on administration of aqueous decoctions of the plant extract. Crude ethanolic extract has also been shown to possess blood glucose lowering effect in diabetic rats after 2 weeks of administration (1). Alcoholic leaf extract of AML in the dose of 250 mg/kg orally for a week has shown to have hypoglycemic effect (13). The aqueous and alcoholic extracts demonstrated hypoglycemic effects 1–5 hours after oral administration (14).

AML treated animals also demonstrated decreased rates of protein catabolism when compared to untreated diabetic animals. However, the rate of protein catabolism was still higher than controls.

There is also an increased oxidative stress in diabetic rats as evidenced by higher MDA and lower GSH levels compared to controls. On administration of AML extract, the MDA levels have decreased and the GSH levels have increased. This indicates that in the presence of AML there is an improvement in the oxidative stress. Increased oxidative stress in the tissues and blood of streptozotocin diabetic rats was similarly reported. This was said to be a contributory factor in the development of the complications of diabetes (15, 16). It was observed in that study that GSH administration reverses these effects (16). In vitro studies have demonstrated that MDA level in RBC's began to rise after the oxidation of RBC GSH. Presence of MDA also decreased fluidity of RBC membrane (17).

Similar improvement in the antioxidant status in diabetes was reported by administration of *Coccinia indica* leaf extract (18), *Musa sapientum* flower extract (19), *Tinospora cordifolia* root extract (20) and *Phaseolus vulgaris* pod extract (21). The fruit extract of Aegle marmelos was also shown to have antioxidant effect in plasma at a dose of 250 mg/kg body weight. It was reported to be more effective than glibenclamide in restoring the antioxidant parameters (22).

Plasma GST levels were higher in diabetic rats than in controls. On AML, there was a fall in plasma GST levels, however they did not approach control levels. There are no reports available on the effect of AML extract on plasma GST. In mice, AML is reported to induce the phase II enzymes of detoxification ie, GST in liver (23). In diabetes mellitus, the plasma GST may increase to combat the enhanced oxidant stress. Administration of AML has lowered the oxidant stress and thus there may be an adaptive lowering of plasma GST levels in the group III animals.

The aqueous extract of AML has shown a hypoglycemic effect with maximal effect at the end of 2nd week. This would be useful in assessing the effect of the drug in diabetic patients after oral administration for two weeks. We were also able to demonstrate that it offers significant protection against oxidative stress. This would be useful as free radicals are reported to have a causative role in the development of complications of diabetes. Herbal formulations with a simultaneous antioxidant effect, would thus be more useful in the management of diabetes mellitus. Their use alone or in combination with oral hypoglycemic agents or insulin may help in the better control of blood glucose level in

diabetic subjects. Also, measurement of GSH levels in RBC may reflect the intracellular GSH status in the various organs. It may thus be used as a parameter to assess oxidative stress in treated diabetic individuals.

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