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ANTIDIABETIC ACTIVITY OF *AEGLE MARMELOS* AND ITS RELATIONSHIP WITH ITS ANTIOXIDANT PROPERTIES

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Abstract : Oxidative stress induced by alloxan has been shown to damage pancreatic β-cell and produce hyperglycemia in rats. *Aegle marmelos* leaf extract is being used in Ayurveda as a medicine for diabetes. The present study examined the action of *Aegle marmelos* against experimental diabetes as well as the antioxidant potential of the drug. A methanolic extract of *Aegle marmelos* was found to reduce blood sugar in alloxan diabetic rats. Reduction in blood sugar could be seen from 6th day after continuous administration of the extract and on 12th day sugar levels were found to be reduced by 54%. Oxidative stress produced by alloxan was found to be significantly lowered by the administration of *Aegle marmelos* extract. This was evident from a significant decrease in lipid peroxidation, conjugated diene and hydroperoxide levels in serum as well as in liver induced by alloxan. Catalase and glutathione peroxidase activity in blood and liver were found to be increased from 9th day onwards after drug administration. Superoxide dismutase and glutathione levels were found to be increased only on 12th day. These results indicate that *Aegle marmelos* extract effectively reduced the oxidative stress induced by alloxan and produced a reduction in blood sugar.

Key words : *Aegle marmelos* antidiabetic alloxan antioxidant enzymes hyperglycemia

INTRODUCTION

Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental factors and chemicals. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications in human beings (1). Oxidative stress in diabetes has been shown to co-exist with a reduction in the antioxidant status. The exact role of oxidative stress in the etiology of human diabetes is however not known. Oxidative stress has been shown to produce glycation of proteins, inactivation of enzymes, alterations in structural functions of collagen basement membrane (2). Oxidative stress may have significant effect in the glucose transport protein (GLUT) or at

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insulin receptor (3). Scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes and may alleviate the diabetes as well as reduce its secondary complications. In the preliminary study we have found that *Aegle marmelos* extract, which is being used in the traditional medicine to reduce the serum glucose level has significant antioxidant activity *in vitro* (4). Alloxan, which is an accepted model for the induction of diabetes, has been shown to damage islet cells of pancreas by the liberation of oxygen radicals (5). In the present study we evaluated the effect of *Aegle marmelos* on blood sugar levels and markers of oxidative stress (i.e. lipid peroxidation, conjugated diene and hydroperoxide levels in serum, and catalase, glutathione and superoxide dismutase in blood and liver) in alloxan treated rats.

METHODS

Preparation plant extract

The leaves of *Aegle marmelos* were collected around Thrissur and identified against voucher specimen and kept at Amala Ayurvadic Centre, Thrissur. Leaves were dried at 50°C, powdered coarsely and extracted with 75% methanol twice by soaking in a container overnight. The extract was evaporated to dryness under vaccum. Extract was resuspended in distilled water and used for the animal experiments. The yield of the extract was 8.6%.

Animals, drug and chemicals

Male 'Wistar' strain rats weighing 250–300 g were used for the experiment. They were housed in polypropylene cages in air-conditioned room and were allowed free access to drinking water and basal diet. All the animal experiments were approved by Institutional Animal Ethics Committee and were done as per their guidelines.

Alloxan was purchased from Sigma chemicals (St. Louis, Mo, USA). Nitroblue tetrazolium, oxidised glutathione and deoxy ribose were purchased from SRL chemicals, Mumbai. All other chemicals used were of analytical reagent grade.

Experimental procedure

The animals were divided into three groups. Group I, normal controls (n = 6) were fed with normal diet. Group II, animals (n = 30) received freshly prepared alloxan in normal saline intraperitonially as a single dose of 120 mg/kg body weight. Group III, animals (n = 30) were treated with alloxan as in group II. They were fed 100 mg/kg, b.wt of *A. marmelos* extract orally (once daily) suspended in distilled water, starting from the day of alloxan treatment till the completion of the experiment. This dosage was determined from our previous studies (4).

Group I animals (normal) were sacrificed on 3rd day. Animals from group II and III, were sacrificed on 1, 3, 6, 9 and 12th day by pentathol sodium anesthesia. Blood which was collected by heart puncture was partially used to separate serum and partially for erythrocyte preparation. Livers were washed thoroughly with cold saline and kept at –20°C till the analyses were completed.

Biochemical analysis

Serum glucose levels were estimated by GOD/POD enzymatic method of Trinder (6). The levels of lipid peroxidation (LPO) products as thiobarbituric acid reactive substances in liver was estimated by the method of Ohkawa et al. (7) and the serum lipid peroxidation was estimated by the method of Satoh (8), Hydroperoxide was estimated by the method of Recknagel and Ghoshal (9) and conjugated diene was estimated by the method of Buege and Aust (10). Glutathione was estimated both in blood and liver tissue by the method of Moron *et al* (11) based on the reaction of GSH with dithiobis trinitro benzoic acid (DTNB).

Erythrocytes were prepared by the method of Minami and Yoshikawa (12) and superoxide dismutase was estimated by the modified method of McCord and Fridovich (13). Catalase was estimated in the erythrocytes and liver tissue by the method of Aebi (14) by measuring the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm. Serum and tissue glutathione reductase was estimated by the method of Racker (15) where the amount of NADPH consumed during the conversion of GSSG to GSH was measured Glutathione peroxidase was estimated by the method of Paglia and Valentine (16) which is based on the degradation of H_2O_2 in the presence of GSH. The protein content of the enzyme was determined by Lowry's method (17). Haemoglobin content of the blood was estimated by Drabkin's method (18).

Statistical analysis

Statistical analysis was done using the Student 't'-test for glucose estimation and the *in vitro* antioxidant data were analyzed as per Bartett's test of ANOVA using one way classification.

RESULTS

Effect of *Aegle marmelos* extract on serum glucose level

Glucose levels were found to be significantly increased till 9th day after alloxan administration and there after decreased on 12th day. Decrease in serum glucose from 12th day onwards may be due to the regeneration of β cell of the pancreas, which were destroyed by alloxan. Administration of *A. marmelos* extract produced a significant ($P<0.001$) decrease in the serum glucose as compared to diabetic control group from 6th day onwards and reduced to 54% of the initial value of day 12 (Fig. 1).

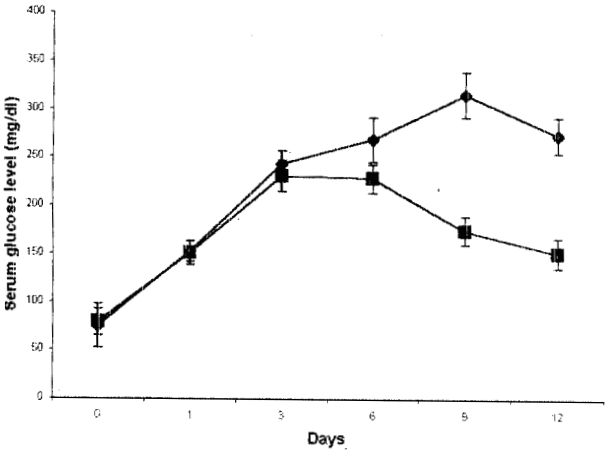

Fig. 1: Effect of *Aegle marmelos* extract on serum glucose level in alloxan induced diabetic rats.
—♦— Alloxan
—■— Alloxan + *Aegle marmelos* extract

Effect of *Aegle marmelos* extract on lipid peroxide, hydroperoxides and conjugated diene levels in serum and liver in diabetic rats

Serum lipid peroxidation level was elevated significantly in diabetic animals compared to normal animals. Administration of *A. marmelos* extract reduced the serum lipid peroxidation significantly ($P<0.001$) from 9th day. The liver lipid peroxidation in diabetic group was found to be increased from day 3 ($P<0.001$). *A. marmelos* treatment was found to decrease the liver lipid peroxidation level significantly ($P<0.001$). Hydroperoxides in blood and liver were found to be significantly increased in untreated diabetic animals from day 3. Treatment with *A. marmelos* extract reduced the hydroperoxide level both in blood and liver from day 3. Conjugated dienes were significantly ($P<0.001$) increased in diabetic rats, which were almost double in blood from day 9, when compared to normal rats. Administration of *A. marmelos* extract significantly decreased ($P<0.001$) conjugated diene in blood and liver from 3rd day onwards and values were similar to that of normal from 9th day (Table I).

TABLE I: Effect of *Aegle marmelos* on lipidperoxidation (LPO), hydroperoxide (HP) and conjugated diene (CD) in blood and liver tissue in alloxan induced diabetic rats.

		Normal	*Alloxan*	*Alloxan+A. marmelos*
LPO Serum	A	1.27 ± 0.13^{b}	$1.41\pm0.14^{a*}$	$1.14\pm0.05^{c*}$
	B	–	$1.65\pm0.22^{a**}$	$1.29\pm0.11^{b**}$
	C	–	$1.93\pm0.42^{a**}$	$1.11\pm0.07^{b**}$
	D	–	$1.81\pm0.37^{a*}$	$0.96\pm0.09^{c**}$
LPO Liver	A	0.829 ± 0.03^{b}	$0.862\pm0.03^{a*}$	$0.801\pm0.02^{c*}$
	B	–	0.898 ± 0.02	$0.779\pm0.02^{b**}$
	C	–	$0.921\pm0.02^{a**}$	$0.756\pm0.02^{b**}$
	D	–	$0.909\pm0.02^{a**}$	$0.733\pm0.03^{c**}$
HP Blood	A	0.94 ± 0.05^{b}	$1.81\pm0.21^{a*}$	$1.03\pm0.05^{b**}$
	B	–	$2.46\pm0.18^{a**}$	$1.10\pm0.05^{b**}$
	C	–	$2.69\pm0.17^{a**}$	$0.98\pm0.09^{b**}$
	D	–	$2.59\pm0.17^{a**}$	$0.89\pm0.05^{b**}$
HP Liver	A	10.01 ± 0.90^{b}	$19.02\pm3.23^{a**}$	$11.08\pm0.41^{b**}$
	B	–	$26.57\pm4.69^{a**}$	$11.28\pm0.29^{b**}$
	C	–	$27.99\pm4.95^{a**}$	$10.42\pm0.26^{b**}$
	D	–	$25.36\pm5.72^{a**}$	$9.58\pm0.21^{b**}$
CD Blood	A	0.81 ± 0.03^{b}	$1.02\pm0.07^{a**}$	$0.89\pm0.02^{b**}$
	B	–	$1.71\pm0.24^{a**}$	$0.91\pm0.05^{b**}$
	C	–	$2.18\pm0.12^{a**}$	$0.89\pm0.04^{b**}$
	D	–	$2.06\pm0.09^{a**}$	$0.84\pm0.05^{b**}$
CD Liver	A	8.10 ± 0.65^{b}	$11.95\pm0.47^{a**}$	$10.59\pm0.49^{a**}$
	B	–	$14.69\pm0.61^{a**}$	$12.08\pm0.67^{a**}$
	C	–	$15.94\pm0.34^{a**}$	$9.15\pm0.62^{b**}$
	D	–	$15.10\pm0.62^{a**}$	$8.11\pm0.52^{b**}$

A—day 3; B—day 6; C—day 9; D—day 12. Units: LPO serum—n mol/ml, LPO liver—n mol/mg protein; HP blood—U/g Hb, HP liver—mm/100 g tissue, CD blood—U/g Hb, CD liver—mm/100 g tissue.
Alphabets (a, b, c) indicate the result of ANOVA test. The alloxan treated group was compared with normal. Where as, *A. marmelos* treated group with the alloxan treated and normal group. Significant at **$P<0.001$, *$P<0.05$ levels.

Effect of *A. marmelos* extract on glutathione (GSH) content

The GSH level in erythrocytes was found to be low in alloxan treated group till day 9. In the drug treated group, GSH levels were low on 3rd day and the values increased significantly on 9th day. GSH level in the liver did not show any significant changes upon treatment with *A. marmelos* extract when compared with untreated diabetic animals (Table II).

Effect of *A. marmelos* on *in vivo* antioxidant enzymes

The superoxide dismutase activity was found to be reduced in erythrocytes of animals treated with alloxan. SOD values in rats treated with alloxan along with *A. marmelos* were significantly higher (P<0.001) on all days. SOD in the liver was also found to be low after alloxan treatment. This value was found to be higher in animals treated with the *A. marmelos* extract.

TABLE II: Effect of *Aegle marmelos* on lglutathione (GSH), superoxide dismutase (SOD) and catalase in blood and liver tissue in alloxan induced diabetic rats.

		Normal	*Alloxan*	*Alloxan+A. marmelos*
GSH	A	31.8±2.05[a]	30.9±2.00[a]**	26.9±0.85[b]**
Blood	B	–	28.4±1.82	27.2±0.71[b]**
	C	–	26.9±1.73[c]**	28.3±0.29[b]**
	D	–	28.1±1.68[b]**	30.8±1.08[a]**
GSH	A	21.7±2.09[a]	20.0±2.22	18.6±1.76
Liver	B	–	19.5±1.86	17.4±1.68
	C	–	18.6±1.38[b]*	18.1±2.17[b]*
	D	–	19.2±1.32	18.5±1.33
SOD	A	494.3±91.2[a]	459.2±54.4	466.2±20.2
Blood	B	–	439.8±37.9	453.2±25.1
	C	–	292.6±24.6[c]**	353.5±28.1[b]**
	D	–	323.2±17.1[b]**	444.1±21.6[a]**
SOD	A	14.6±1.2[a]	12.6±1.1	12.9±0.9
Liver	B	–	12.2±0.9	10.5±1.3
	C	–	8.1±0.6[b]**	12.9±1.1[a]**
	D	–	8.3±0.6[b]**	13.6±1.4[a]**
Catalase	A	90.6±0.71[a]	80.6±1.20[b]	80.4±0.25[b]
Blood	B	–	50.2±1.48	80.7±10.5
	C	–	30.3±0.36[b]**	90.0±0.35[b]**
	D	–	60.6±1.49[b]**	90.6±0.27[a]**
Catalase	A	8.5±1.50[a]	6.4±0.69[c]**	8.4±0.25[a]**
Liver	B	–	5.9±1.24	8.7±0.15
	C	–	4.5±0.43[c]**	9.0±0.35
	D	–	6.6±1.37[c]**	9.6±0.27[a]**

A—day 3; B—day 6; C—day 9; D—day 12. Units: GSH blood and liver—n mol/ml; SOD blood—U/g Hb, SOD liver—U/mg protein; Catalase blood—k/g Hb, Catalase liver—k/sec/mg protein.
Alphabets (a, b, c) indicate the result of ANOVA test. The alloxan treated group was compared with normal. Where as, *A. marmelos* treated group with the alloxan treated and normal group. Significant at **P<0.001, *P<0.05 levels.

Catalase activity in erythrocytes from diabetic rats was found to be significantly decreased (P<0.001) when compared to normal. Catalase activity was found to be increased in extract treated group and on 12th day it was found to be similar as normal level. Similarly, liver catalase activity, which was lower in diabetic group on 3rd day, was found to be significantly higher in *A. marmelos* treated group (Table II).

The blood glutathione peroxidase was significantly low in diabetic group from 3rd day when compared to normal animals. The values were elevated significantly (P<0.001) by the continuous administration of *A. marmelose* from 3rd day. Liver GPx was found to be low in alloxan diabetic group whereas the levels were elevated significantly (P<0.001) in extract treated group and the values were almost double on day 12. Glutathione reductase in blood and liver were decreased in untreated diabetic animals when compared to normal. Continuous administration of the *A. marmelose* extract showed significant increase in the glutathione reductase level (Table III).

TABLE III: Effect of *Aegle marmelos* on glutathione peroxidase (GPx) and glutathione reductase (GR) in blood and liver tissue in alloxan induced diabetic rats.

		Normal	*Alloxan*	*Alloxan+A. marmelos*
GPx Blood	A	773.9 ± 30.8^{a}	$357.5\pm63.9^{c**}$	$563.6\pm9.1^{b**}$
	B	–	351.2 ± 62.0	582.5 ± 25.4
	C	–	$294.4\pm29.9^{c**}$	$645.6\pm22.1^{b**}$
	D	–	$325.9\pm79.6^{b**}$	$691.9\pm33.4^{a**}$
GPx Liver	A	63.6 ± 9.9^{a}	$28.8\pm8.9^{c**}$	$38.7\pm4.2^{b**}$
	B	–	$20.6\pm2.8^{c**}$	$48.9\pm9.3^{b**}$
	C	–	$27.8\pm6.8^{c**}$	$71.7\pm4.7^{a**}$
	D	–	$36.8\pm4.9^{c**}$	$109.1\pm11.2^{a**}$
GR Blood	A	14.30 ± 0.48^{a}	13.73 ± 0.73	14.03 ± 0.52
	B	–	12.97 ± 0.29	13.18 ± 0.36
	C	–	$12.56\pm0.36^{c**}$	$13.79\pm0.14^{b**}$
	D	–	12.67 ± 0.30	14.17 ± 0.14
GR Liver	A	31.63 ± 0.45^{a}	$23.47\pm3.43^{b**}$	$33.06\pm2.59^{a**}$
	B	–	16.19 ± 2.14	29.31 ± 1.39
	C	–	$21.85\pm4.42^{b**}$	$31.91\pm1.64^{a**}$
	D	–	$22.42\pm5.01^{c**}$	$36.55\pm3.06^{a**}$

A—day 3; B—day 6; C—day 9; D—day 12.
Units: GPx and GR blood—U/1, GPx and GR liver—U/mg protein.
Alphabets (a, b, c) indicate the result of ANOVA test. The alloxan treated group was compared with normal. Where as, *A. marmelos* treated group with the alloxan treated and normal group. Significant at **P<0.001 levels.

DISCUSSION

Present study indicates that 75% methanolic extract of *A. marmelose* (100 mg/kg b.wt) significantly decreased serum glucose level in hyperglycaemic animals. Alloxan administration produced, elevated level of lipid peroxidation, hydroperooxides and conjugated diene that is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage. Karpen *et al* (19) observed an elevated level of lipid peroxides in the plasma of streptozotocine diabetic rats and lipid peroxidation is one of the characteristic features of chronic diabetes (20). The significant decline in the concentration of these constituents in the liver tissue and serum of *A. marmelose* treated diabetic animals indicate that *A. marmelose* extract effectively increased antioxidant potential *in vivo*.

GSH is a major non-protein thiol in living organisms, which plays a central role in co-ordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. SOD, CAT and GPx constitute a mutually supportive team of defense against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of O_2–. In hyperglycaemia, glucose undergoes autooxidation and produces superoxide and it produces free radicals that inturn leads to lipid peroxidation in lipoproteins. CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of H_2O_2 to water and oxygen and thus protect the cell from oxidative damage produced by H_2O_2. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In our study, decline in the activities of these enzymes in alloxan-induced animals and attainment of near normalcy in *A. marmelose* treated rats indicate oxidative stress elicited by alloxan had been nullified due to the effect of the extract. This observation perfectly agrees with those of Krishnakumar *et al* (21) who demonstrated hypoglycaemic and antioxidant activity of *Salacia oblonga*. Wall extract in streptozotocin induced diabetic rats.

Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore and optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *A. marmelose* can rightly be mentioned as a plant of considerable interest.

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