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RESEARCH ARTICLE

Hypoglycemic and Hypolipidemic Effect of *Aegle marmelos* Leaf Extract in Alloxan Induced Diabetic Male *Albino* Rats

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ABSTRACT

Aegle marmelos is used extensively in the indigenous system of medicine as an anti-diabetic agent. The current investigation focuses on the serum insulin augmentation, anti-hyperglycemic and anti-hyperlipidemic property of aqueous leaf extract of *Aegle marmelos* on alloxan induced diabetic male albino rats. The diabetes induced animals were fed with the leaf extract at 250mg/kg body wt and 350mg/kg body wt. The aqueous leaf extract administrated animals revealed a significant increment of serum insulin levels, higher reduction in hyperglycemia and hyperlipidemia when compared to the diabetic control rats. These results further suggest that *Aegle marmelos* may be useful in the therapy and management of hyperglycemia by reducing blood glucose and hyperlipidemia by reducing lipid levels.

KEYWORDS

Aegle marmelos, Alloxan, Hyperglycemia, Hyperlipidemia

INTRODUCTION

Diabetes mellitus (DM) is one of the major metabolic disorders currently associated with considerable morbidity, mortality and several long term complications in the affected individuals.

Hyperglycemia is caused either by insufficient insulin secretion or insulin resistance. The percentage of people affected by DM was rapidly rising in India. At present more than 40 million people are affected in India alone which represent nearly 20 of total diabetes population worldwide[1].

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The concentration of glucose in blood depends on its production and utilization in normal and diabetic condition by different tissues. Absence or insufficiency of insulin leads to overproduction of glucose and its underutilization by the liver. However, the insulin-independent tissues such as kidney show overutilization of glucose during diabetes because of hyperglycemia[2,3]. Hyperglycemia directly contributes to the complications through increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure. Diabetic complications include specific microvascular complications such as thickening of capillary basement membranes, retinopathy and nephropathy[4,5]. If diabetes is left untreated, it develops into complications such as irreversible hepatic and renal deterioration. A spectrum of hepatic and renal pathological changes along with functional

disturbances has been reported in experimental diabetes[6].

The control of blood glucose in diabetic patients was achieved mainly by the use of oral hypoglycemic/antihyperglycemic agents and insulin. However, all these treatments have limited efficacy and have been reported to be associated with undesirable side effects. In order to overcome the side effects associated with diabetes, interest has shifted to the use of other alternative medicines[1]. Plants with antidiabetic activities are important sources for the development of new drugs in the treatment of DM. In recent years, considerable attention has been focused on an intensive search for a novel type of antihyperglycemic agent from numerous plant materials. The use of medicinal plants has flourished as an alternative for the treatment of diabetes because modern medicines have several side-effects and are expensive. A multitude of herbs and medicinal plants have been described for the treatment of diabetes throughout the world as they might provide a basis for new synthetic antidiabetic analogues with potent activity[7].

Aegle marmelos (Family:Rutaceae) is a traditional medical plant, the root is sweet; cures fever, pain in the abdomen, palpitation of the heart and urinary problems. The leaves are astringent, digestive, laxative and useful in dysentery. The ripe fruit is a restorative tonic, astringent, laxative, good for the heart & brain[8].

In the present study, we investigated the possible antihyperglycemic activities of the aqueous extract obtained from the leaves of *Aegle marmelos* in two different dosages (250mg/kg and 350mg/kg of body wt) in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Material

The leaves of *Aegle marmelos* (Family-Rutaceae) were collected in and around Vellore District, Tamilnadu, India. The leaves were cleaned with distilled water and shade dried at room temperature and authenticated in the Department of Botany, C Abdul Hakeem College, Melvisharam, Vellore District, Tamilnadu, India.

Preparation of Plant Extract

About 100 gms of dried powdered leaves of *Aegle marmelos* were taken and mixed with 500 ml of distilled water and magnetically stirred in a separate container for overnight at room temperature. The residue was removed by filtration and the aqueous extracts were concentrated under vacuum to get solid yield of 7%. The plant extract was administered orally to animals in aqueous solution[9].

Animals

Male *albino* rats weighing around 180-200 gms were purchased from Tamilnadu Veterinary and Animal Science University, Chennai, India. The animals were kept in polypropylene cages and maintained in an animal room, under controlled temperature of 25±2ºC.Humidity and airflow conditions with a 12±1 hr light and dark schedule were maintained in the animal house till the animals were acclimatized to the laboratory conditions, They were fed with commercially available rat chow and had free access to water. The experimental protocol was conducted in accordance with the institutional guideline[9].

Experimental Induction of Diabetes

Diabetes was induced in the rats by the administration of single intraperitoneal dose of alloxan monohydrate (150mg/kg body wt) (SD Fine Chem. Limited, Mumbai) in normal saline (10).Two days after alloxan injection, rats were screened for diabetes. All animals were allowed free access to water and pellet diet and they were maintained at room temperature in polypropylene cages.

Experimental Design

Group I: Normal rats.

Group II: Diabetic control rats (Alloxan induced).

Group III: Diabetic induced animals fed with aqueous leaves extract of *Agele marmelos* (250 mg/kg body wt) for 30 days.

Group IV: Diabetic induced animals fed with aqueous leaves extract of *Aegle marmelos* (350 mg/kg body wt) for 30 days.

In each group six animals were maintained for 30 days. The body weight and blood glucose were measured daily. After the experimental period the normal, diabetic control and plant treated animals were anesthetized and sacrificed. The serum was separated for biochemical estimation.

Biochemical Analysis

Blood glucose level: The blood was collected from the tip of the tail vein from the rats and the blood glucose was measured using Gluco Chek glucose estimation kit (Aspen diagnostic (P) Ltd. Dehil, India).

Estimation of Plasma Insulin Levels

Plasma insulin was estimated using radio immuno assay (RIA) kit supplied by Linco research Inc, Stat diagnostic, Mumbai, India.

Estimation of Lipid Profile in Blood Samples

On completion of the treatment, blood samples were collected and lipid profiles for all groups of animals were measured using commercially available kits. Total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) cholesterol levels in serum were determined according to the instruction of the manufacturer (Transasia Bio Medical Limited, Mumbai, India). For the determination of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol Friedewald's formula which states: VLDL cholesterol = Triglycerides/5 and LDL cholesterol = Total cholesterol – (VLDL + HDL cholesterol) was used[11].

Statistical Analysis

The results were expressed in Mean ±SEM. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

RESULTS

The hypoglycemic effect of aqueous extract of *Aegle marmelos* was investigated in alloxan induced diabetic rats. The body weights of the control group decreased significantly by 18.42% when compared to the normal groups. In aqueous extract treated group, the body weight increased significantly by 5.81 % in 250 mg/kg body wt and 11.50% in 350 mg/kg body wt. The insulin levels were tested in normal, control and plant treated groups. In group II animals, the level of insulin was significantly reduced by 83.52% when compared to the group I (normal rats). In extract treated rats, the levels of insulin were found to have increased significantly by 275.47 % in 250 mg/kg body wt and 363.16 % in 350 mg/kg body wt when compared to the level of control group. The blood glucose levels of group II animals were tested. The level of glucose was increased by 278.49% when compared to the normal group. The blood glucose level was reduced in extract treated rats (25.07 % in 250mg/kg and 49.20% in 350 mg/kg body wt) when compared with the diabetic control rats. The highest reduction was recorded at 350 mg/kg body wt (Table-1)

Lipid profile

The lipid profile such as TC, TG, HDL, LDL and VLDL were tested in normal, control and plant extract treated groups. The levels of TC, TG, LDL and VLDL were significantly increased in control group when compared to the normal group. In aqueous extract treated group there were significant reductions of TC (43.23%), TG (42.81%), LDL (63.91%), and VLDL (42.77%), when compared to the control group. In addition, a significant increase in the level of HDL (115.79%) in the plant extract treated diabetic rats was seen. In the case of untreated diabetic rats, there was a fall in HDL level (65.24%). A more effective reduction of TC, LDL, VLDL and TG was recorded at the doses of 350 mg/kg than 250 mg/kg body wt (Table-1).

DISCUSSION

In this study the aqueous leaf extract of *Aegle marmelos* at different doses produced a significant fall in the blood glucose level and lipid levels in diabetes induced rats.

Table 1: Effect of the aqueous extract of *Aegle marmelos* on body weight (gms), serum insulin (µu/ml), Blood glucose (mg/dl) and Lipid profile (mg/dl) in alloxan induced diabetic rats.

Parameters	Normal rats (N)	Diabetic control rats (C)	% of changes N vs C	Plant extract treated groups				* P values
				250mg/kg body wt	% of changes C vs 250mg/kg body wt	350mg/kg body wt	% of changes C vs 350mg/kg body wt	
Body weight (gms)	190 ± 4.87	155 ± 2.81	-18.42	164.00 ± 3.60	5.81	172.83 ± 2.74	11.50	0.001
Serum insulin	57.66 ± 1.36	9.50 ± 0.76	-83.52	35.67 ± 2.16	275.47	44.00 ± 1.93	363.16	0.001
Blood glucose	99.50 ± 1.57	376.6 ± 4.40	278.49	282.17 ± 4.36	-25.07	191.33 ± 2.72	-49.20	0.001
Lipid profile								
TC	124.83 ± 1.64	231.33 ± 1.81	85.32	219.17 ± 3.47	-5.26	131.33 ± 3.18	-43.23	0.001
TG	100.83 ± 1.81	285.6 ± 3.22	183.25	192.50 ± 3.82	-32.60	163.33 ± 4.29	-42.81	0.001
HDL	54.66 ± 1.25	19.00 ± 1.52	-65.24	33.17 ± 2.26	74.58	41.00 ± 1.53	115.79	0.001
LDL	46.66 ± 2.03	155.16 ± 2.36	232.53	144.50 ± 3.09	-6.87	56.00 ± 2.13	-63.91	0.001
VLDL	25.33 ± 1.23	57.66 ± 1.3	127.64	36.00 ± 1.93	-37.57	33.00 ± 1.53	-42.77	0.001

Each value represents six individual observations. Mean ± SEM, '+', '-' indicate percent increase or decrease over control. 'P' denotes the statistical significance and '*P' denotes statistical significance of ANOVA, to test the difference between the experimental groups, TC- Total cholesterol, TG- Triglyceride, HDL -High density lipoprotein, LDL- Low density lipoprotein, VLDL- Very low density lipoprotein.

Alloxan causes massive reduction in insulin release, through the destruction of ß-cells of the islets of Langerhans. In our study, we have observed a significant increase in the plasma insulin level when alloxan diabetic rats were treated with aqueous extract of *Aegle marmelos*. This could be due to the effect of the extract on the ß-cell of the islets of langerhans by stimulating them to produce insulin. The significant and consistent antidiabetic effect of plant extract in alloxan diabetic rats may also be due to enhanced glucose utilization by peripheral tissues[12].

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease.

This abnormally high level of serum lipid is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities[13, 14, 15].

The blood glucose level of plant extract fed animal was significantly reduced. The highest depletion was recorded in the 350mg/kg body wt., dosage rats. The levels of serum TC, TG, LDL, and VLDL were found to be significantly

reduced in the plant extracts treated diabetic animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk[16].

CONCLUSION

In conclusion the present study reveals the antidiabetic and antilipidimic potential of aqueous leaf extract of *Aegle marmelos* leaves. The results were compared with that of the diabetic control group. The findings suggest that the aqueous leaf extract of *Aegle marmelos* has a potent hypoglycemic and hypolipidemic property as it significantly reduced the blood sugar level and lipid levels in alloxan induced diabetic rats as compared to diabetic control group.

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