

HYPOLIPIDEMIC EFFECT OF *AEGLE MARMELLOS* LEAF EXTRACT IN STREPTOZOTOCIN (STZ) INDUCED DIABETIC MALE *ALBINO* RATS

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ABSTRACT: *Aegle marmelos* (vilvam in Tamil) leaves are used extensively in the indigenous system of medicine as an anti-diabetic agent. The current investigation focuses on the lipid lowering property of an aqueous extract of *Aegle marmelos* leaves on streptozotocin (STZ) induced diabetic rats. The lipid profiles such as serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), and very low density lipoprotein (VLDL) were studied. Extracts were administered orally at increasing dose levels of 250mg, 350mg, 450mg/kg body wt., to STZ induced diabetic rats. The levels of TC, TG, LDL, HDL, and VLDL were found to be reduced significantly ($P < 0.001$) when compared to that of diabetic control rats. These results further suggest that *Aegle marmelos* may be useful in the therapy and management of hyperlipidemia by reducing lipid levels.

KEY WORDS: *Aegle marmelos*, streptozotocin (STZ), diabetes, hyperlipidemia

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders with one common manifestation of hyperglycemia. The disease crosses many boundaries, from physicians to geneticist, and represents a major health problem. Diabetes means chronic excretion of excessive volume of the urine and mellitus means honey. So the word diabetes mellitus refers to chronic excretion of large volume of urine containing glucose.

The cardinal manifestation of diabetes mellitus is hyperglycemia, which results from 1. Decreased entry of glucose into the cells, 2. Decreased utilization of glucose by various tissues and 3. Increased production of glucose (Gluconeogenesis) by the liver. Diabetes is characterized by symptoms such as weakness, poly-urea, excessive thirst as well as ketonemia, ketonuria

and ketosis due altered metabolism of lipids and proteins.

Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipids, carbohydrates and proteins, in addition to damaged β -cells of pancreas and increased risk of complications of vascular diseases (1).

In 2006 according to the World Health Organization, at least 171 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double. Diabetes mellitus occurs throughout the world, but is more common in the more developed countries.

The prevalence of diabetes and the wide ranging nature of its complications, present a challenge to those scientists of the disease who wish to assimilate the most recent clinical and scientific advantages (2).

In a diabetic condition, increased serum lipids are due to the increased lipolysis of adipose tissue (3), and thereby abnormal lipoprotein concentration. The low HDL cholesterol and high VLDL lipoproteins cause atherosclerosis (4), and renal damage (5).

A number of pharmacological and chemical agents act as diabetogenic and produce variety of diabetic complications. Streptozotocin induced diabetes is an experimental model widely used to study glycemic and lipidemic changes in plasma (6,7). Many species of plants and herbs are known to act as anti-diabetic agents, but only a few of them have been investigated (8,9).

Aegle marmelos (Family: *Rutaceae*) is a traditional medicinal plant. The root is sweet; cures fever, pain in the abdomen, palpitation of the heart, urinary problems. The leaves are astringent, digestive; laxative, when fresh; the flowers allay thirst and vomiting; useful in dysentery. The ripe fruit is a restorative tonic, astringent, laxative; good for the heart and brain (10).

The present study was carried out in male albino rats to test the efficacy of aqueous leaf extract of *Aegle marmelos* on serum lipid profile changes associated with streptozotocin induced diabetes.

MATERIALS AND METHODS

PLANT MATERIAL

The leaves of *Aegle marmelos* L. (*Rutaceae*) were freshly collected in and around Vellore District, Tamilnadu, India. The leaves were cleaned and shade dried at room temperature and authenticated and a voucher specimen (No:VCV/2/2007) is kept at the Department of Botany, Voorhees College, Vellore – 632 001, Tamilnadu, India.

PLANT EXTRACTS PREPARATION

100gms of powdered leaves of the plant were taken and mixed with 500ml of distilled water and magnetically stirred in a container overnight at room temperature. The residue was removed by filtration and the aqueous extracts were concentrated under vacuum to get solid yield of 10%.

The plant extract was administered to animals in aqueous solution.

ANIMALS

Adult male albino rats of Wistar strain weighing around 180-200g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of

25±2°C and 55-65% relative humidity. A 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

EXPERIMENTAL INDUCTION OF DIABETES

Diabetes was induced in the overnight fasted animals by a single intra peritoneal injection of freshly prepared solution of streptozotocin (STZ) (Sigma, USA) 35mg/kg body weight in 0.1M cold citrate buffer pH 4.5. (11,6,7). The animals were allowed to drink 5% glucose solution to overcome the drug-induced hypoglycemia. The control rats were injected with citrate buffer alone as placebo. The animals were considered diabetic if the blood glucose values were >250mg/dl on the third day after STZ injection.

EXPERIMENTAL DESIGN

The studies were conducted in the four groups of animals

Group I: Normal rats.

Group II: Diabetic (STZ induced) control rats.

Group III: Short term (ST): Diabetic animals kept for 7days.

Group IV: Long term (LT): Diabetic animals kept for 25 days.

The diabetic animals of both groups (ST and LT) were fed with plant extract in increasing dosages of 250mg, 350mg, and 450mg/kg body weight to assess therapeutic effect of the extracts. Separate batches were maintained in each group for each dose level.

BIOCHEMICAL ANALYSIS

The biochemical estimation was carried out in our lab by using the following methods. Serum TC (12), TG (13), LDL, HDL, VLDL (14).

STATISTICAL ANALYSIS

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

RESULT

Tables 1-5 show the effect of oral administration of aqueous leaf extract of *Aegle marmelos* on lipid profile in STZ induced diabetic rats.

The lipid profiles such as TC, TG, LDL and VLDL levels were increased significantly in the short term and long term diabetic control rats (DC) when compared to that of normal rats. Where as HDL levels

were decreased significantly in diabetic control rats (DC) when compared to that of normal rats.

The aqueous extract of *Aegle marmelos* was administered orally at increasing dose levels of 250mg, 350mg and 450mg/kg body wt., to diabetic rats of both the short term and long term groups. In the short term group diabetic animals at 250mg/kg dosage recorded a non significant change in the TC, TG, HDL, LDL and VLDL levels. On the other hand when the dosage levels were increased to 350mg and 450mg/kg body wt., a significant ($P<0.001$) depletion in the total cholesterol level was recorded in the short term and also in the long term diabetic animals. The depletion in the total cholesterol was dose dependent and the highest reduction in the cholesterol was recorded 34.15% in 450mg/kg body wt., in the long term plant extract fed animals when compared to diabetic control animals.

The depletion of triglycerides recorded 10.20 % for short term diabetic animals at 450mg/kg body wt., at the same time the highest depletion of 35.50% was recorded in the long term diabetic animals. The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly ($P<0.001$) after the administration of the plant extract. The highest increment was recorded at 450mg/kg body wt., dosage level in both short term and long term groups. The highest increment of the HDL recorded was 36.20% in long term group animals.

The plant extract fed groups of both long term and short term recorded a significant decrement ($P<0.001$) in the levels of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) when compared to the diabetic animal groups. The decrement of both LDL and VLDL in extract fed animals was dose dependant, and the highest depletion was recorded at 450mg/kg body wt., extract dose level i.e. 34.78%, 37.93% in long term diabetic animal groups.

DISCUSSION

Premature and extensive atherosclerosis involving renal, peripheral and cardiovascular sites remain major complications of diabetes mellitus. In addition to hyperglycemia, systemic or local elevations in insulin may contribute to aberrant lipid metabolism and vascular wall function (15).

Since alteration in serum lipid profiles are known in diabetics, which are likely to increase the risk of

coronary heart disease (16,17,18), a reduction in serum lipids, particularly LDL and VLDL fractions and triglycerides levels should be considered as beneficial in the long term prognosis of the patients.

The diabetogenic effect of Streptozotocin was utilized to induce hyperglycemia in the present study. Nitric oxide has been demonstrated to participate in the beta cell damage during Streptozotocin (STZ) induced diabetes. (19,20).

Diabetes affects both glucose and lipid metabolism (21). In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism (22). The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes (23).

The lipoprotein levels in the STZ induced diabetic rats in the present study reveal a significant deranged lipoprotein. The serum total cholesterol content was increased significantly in diabetic animals. The elevated triglycerides in diabetic animals might be due to the consequence of increased synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver and diminished catabolism (24). Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (25,26). The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (25). The high-density lipoprotein (HDL) was significantly reduced in the diabetic rats, which indicate a positive risk factor for atherosclerosis (27).

The levels of serum total cholesterol, triglycerides, LDL, and VLDL were 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 animals. The high-density lipoprotein (HDL) was significantly ($P<0.001$) increased in the plant extract treated animals indicating a reversed atherogenic risk.

Effect of the aqueous leaf extract of *Aegle marmelos* on serum lipid profile levels (mg/dl), in STZ induced diabetic animals.

TABLE – 1: Total cholesterol

Experiments	Diabetic		Plant extract treated		
	Normal (N)	control (DC) (% changes N vs DC)	250mg/kg bd wt., (% changes DC vs 250mg)	350 mg/kg body wt., (% changes DC vs 350mg)	450 mg/kg body wt., (% changes DC vs 450mg)
Short term	79±2.02	86±3.03	81.02±2.03	80.01±1.29	78.81±1.26
% of changes		+8.83*	-5.81*	-6.97*	-8.12*
Long term	79±2.02	123.05±2.10	89.12±1.45	86.05±2.06	81.03±1.89
%of changes		+55.69*	-27.64*	-30.10*	-34.15*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001, NS- Non significant.

TABLE – 2 Triglycerides

Experiments	Diabetic		Plant extract treated		
	Normal (N)	control (DC) (% changes N vs DC)	250mg/kg bd wt., (% changes DC vs 250mg)	350 mg/kg body wt., (% changes DC vs 350mg)	450 mg/kg body wt., (% changes DC vs 450mg)
Short term	85.16±1.41	98.18±1,26	94.12±2.61	90.22±2.16	88.03±2.1
% of changes		+ 15.29*	-4.08*	-8.16*	-10.20*
Long term	85.16±1.41	138.13±2.1	96.38±2.05	92.14±2.87	89.13±1.81
%of changes		+62.35*	-30.43*	-33.29*	-35.5*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001 NS- Non significant.

TABLE – 3: Serum high density lipoprotein (HDL)

Experiments	Diabetic		Plant extract treated		
	Normal control (N)	(DC) (% changes N vs DC)	250mg/kg bd wt., (% changes DC vs 250mg)	350 mg/kg body wt., (% changes DC vs 350mg)	450 mg/kg body wt., (% changes DC vs 450mg)
Short term	30.03±1.41	26.10±1.31	26.07±2.03	28.26±2.68	30.21±1.87
% of changes		-13.3*	-0.11 NS	+8.72*	+15.77*
Long term	30.03±1.41	21.04±1.52	22.79±1.87	25.66±2.12	28.62±1.89
%of changes		-62.35*	+8.52*	+22.20*	+36.20*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001, NS - Non significant.

TABLE – 4: Serum low density lipoprotein (LDL)

Experiments	Diabetic		Plant extract treated		
	Normal control (N)	(DC) (% changes N vs DC)	250mg/kg bd wt., (% changes DC vs 250mg)	350 mg/kg body wt., (% changes DC vs 350mg)	450 mg/kg body wt., (% changes DC vs 450mg)
Short term	32.05±1.79	40.12±2.10	38.62±3.20	34.67±2.67	31.31±2.46
% of changes		+18.75*	-6.5 NS	-16.5*	-23.25*
Long term	32.05±1.79	46.04±2.01	40.21±2.61	36.18±2.26	32.61±2.42
%of changes		+43.75*	-13.50*	-21.74*	-34.78*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001, NS - Non significant.

TABLE – 5: Serum very low density lipoprotein (VLDL)

Experiments	Diabetic		Plant extract treated		
	Normal control (N)	(DC) (% changes N vs DC)	250mg/kg bd wt., (% changes DC vs 250mg)	350 mg/kg body wt., (% changes DC vs 350mg)	450 mg/kg body wt., (% changes DC vs 450mg)
Short term	16.25±2.37	24.12±2.8	21.26±2.46	18.07±2.46	15.89±2.12
% of changes		+50.12*	-13.1*	-24.9*	-40.83*
Long term	16.25±2.37	29.12±1.89	27.2±2.12	23.41±3.46	18.61±2.89
%of changes		+81.25*	-7.24 NS	-22.06*	-37.93*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001, NS - Non significant.

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