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Hypoglycemic and Hypolipidemic Effect of *Aegle marmelos* (L.) Leaf Extract on Streptozotocin Induced Diabetic Mice

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Abstract: The aim of this present study was to determine the probable mechanism of action of *Aegle marmelos* plant leaf extract on streptozotocin induced diabetic mice for the treatment of diabetes mellitus. Once the diabetic condition was achieved after injecting the mice with 60 mg kg^{-1} body weight (b.wt.) of streptozotocin, the mice were treated for another 15 days with aqueous extract of leaves. Data showed that 300 mg kg^{-1} b.wt. of extract was most effective in reverting the diabetic mice to normal condition. Different biochemical parameters like glucose tolerance test, lipid profile, glycogen biosynthesis, glucose uptake, differential regulation of glucose homeostatic enzymes like glucose-6-phosphatase, hexokinase and insulin release *in vitro*, clearly demonstrated the hypoglycemic effect in treated animals. The data showed that this plant's leaf extract has a remarkable hypoglycemic and hypolipidemic effects. Further study is needed to isolate the active principles from this plant and understand its molecular mechanism of action.

Key words: Blood sugar, serum lipids, streptozotocin-diabetic mouse, anti-diabetic, hypoglycemic effect, glucose homeostasis

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine disorder that affects more than 100 million people world wide (6% of the population) and by the year 2025 it is expected to effect five times the number of people that is affecting today (Grover *et al*., 2002; Kim *et al*., 2006). Diabetes mellitus is classified as: insulin-dependent diabetes mellitus (DM type 1) and non-insulin dependent diabetes mellitus (DM type 2). About 90% of patients have type 2 DM with insulin resistance playing a key role in the development of the disease (Fuller *et al*., 1980; Kim *et al*., 2006). Insulin resistance includes decreased stimulation of muscle glycogen synthesis, defects in glycogen synthase and hexokinase activity (Berg *et al*., 2001).

In recent years, interest in herbal agents as therapeutic treatment option has increased due to their limited side effects (Dubey *et al*., 1994; Prince *et al*., 1998; Ladeji *et al*., 2003; Rao *et al*., 2003; Maiti *et al*., 2004). Most of these products have been found to be nontoxic in animal studies done so far. Presently, there are some isolated reports where attempts have been made to understand the mode of hypoglycemic activities of some folk medicines in India (Grover *et al*., 2002; Maiti *et al*., 2004).

Aegle marmelos (AM) is a medium sized, armed deciduous tree found wild, especially in dry forests and is also cultivated throughout Indian subcontinent for its fruit. The fruit of these plants have hard shell encasing fleshy mass that contains the seed. Leaves, fruits, stem and roots of this plant have been used in ethno medicines for several medicinal properties: astringent, antidiarrheal, antidysentric, demulcent, antipyretic, antiscourbutic, aphordisiac and as an antidote to snake venom (Khare, 2004). There are several preliminary reports of the roots and leaves of AM as a hypoglycemic agent in traditional medicine (Prakash, 1992; Alam *et al*., 1990; Grover *et al*., 2002; Mukherjee *et al*., 2006). Preliminary report indicates blood glucose lowering activities in green leaves of AM plants (Chakrabarti *et al*., 1960). The alkaloids extract prepared from the leaves of this plant and the crude extract exhibited hypoglycemic effects on alloxinized rats (Ponnachan *et al*., 1993a, b). Aqueous extract of leaves reversed the increase in km value of liver malate dehydrogenase enzyme and also improved the liver and kidney histopathological conditions of streptozotocin treated diabetic rats (Seema *et al*., 1996; Das *et al*., 1996). Recently few reports also demonstrated the hypoglycemic activities of aqueous extracts of fruits of this plant (Kamalakkannan and Prince, 2003, 2004; Kesari *et al*., 2006). However, limited scientific evidence exists to

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validate these claims since there are only few available reports on the exact mode of action of these extracts and pharmacological actions of this plant (Ponnachan *et al*., 1993a, b; Das *et al*., 1996; Seema *et al*., 1996; Sachdewa *et al*., 2001; Upadhya *et al*., 2004; Kesari *et al*., 2006).

The aim of the present study is to understand the application of AM leaf extract for the management of diabetes and to reflect the enzyme activities related to the regulation of blood glucose level in streptozotocin induced diabetic mice.

MATERIALS AND METHODS

Plant material: The plant leaves were collected from area surrounding the campus of Indian Institute of Technology Roorkee during the month of January-March 2005. The plant materials were identified as per the literature of Ayurveda and by local expert of herbal gardens and also confirmed by Dr. H.S. Dhaliwal, Professor of Plant Biotechnology, Indian Institute of Technology Roorkee, Uttarakhand, India.

Preparation of the aqueous extract of the leaves: AM leaves were thoroughly washed with water and dried in shade. Fifty grams of air dried leaves were ground into fine powder and soaked in water for 7-8 h and stirred occasionally. After soaking, the mixture was filtered using Whatmann No. 1 filter paper. The filtrate was centrifuged at 10,000 rpm at room temperature (25°C) and the pellet was discarded. The supernatant was concentrated up to 100 mL on rotavapor under reduced pressure. The concentrated crude extract was lyophilized into powder (3.5 g) and used for the study.

Selection of animals: The study was conducted on 15 matured albino mice purchased from Jamia Hamdared University, New Delhi, five to six weeks of age, weighing 35 g±5, which were housed in colony cages (five mice per cage) at an ambient temperature of 25±2°C with 12 h light and dark cycles. The mice were fed normal diets purchased commercially from the vendors. The animals were allowed to acclimatize to the laboratory environment for 1 week and then randomly divided in to three groups (n = 5 mice per group): Group 1-untreated (control); Group 2-streptozotocin treated (diabetic); Group 3-streptozotocin induced diabetic mice treated with AM extract (treated). After randomization into various groups, the mice were acclimatized for a period of another 2-3 days in the new environment before initiation of the experiment. Animals described as fasting had been deprived of food for at least 16 h but had been allowed free access to drinking water. All the experiments with animals were carried out as per the guidelines of the institutional animal ethical committee.

Induction of diabetes mellitus followed by AM leaf extract treatment: Over night starved experimental mice from group 2 and 3 (as discussed earlier) were injected with streptozotocin at a dose of 60 mg kg^{-1} b.wt. as described by Sanae *et al*. (1998) with slight modifications. The chemical was injected intraperitoneally (i.p.) within 10 min after dissolving in 0.025 M sodium citrate at pH 4.0. The mice in group 1 were injected with sodium citrate buffer as vehicle control. Fasting Blood Glucose (FBG) was estimated at the time of induction of diabetes and Post Prandial Glucose (PPG) was checked regularly until stable hyperglycemia was achieved. The mice exhibiting blood glucose level ~250 mg dL^{-1} were included in the study as stable hyperglycemic animals. Once the stable hyperglycemia was achieved, the mice belonging to group 3 were injected with AM leaf extract for another 15 days while group 1 and 2 mice received only 0.01% ethanol (as vehicle control).

Effect of fasting blood glucose level: Fasting blood glucose was measured after 15 days of treatment with aqueous extract of AM leaf, during which the animals were fed with normal diets. For the determination of fasting blood glucose, on completion of the 15 days of the treatment with AM leaf extract and vehicle control, the mice were fasted over night. The blood was collected from the tip of the tail vein of the overnight fasted mice and the blood glucose was measured using GOD-POD glucose estimation kit according the manufacturer's instructions (Excel Diagnostics Pvt. Ltd., India). The results were expressed in terms of milligrams per deciliter of blood.

Glucose Tolerance Test (GTT): In order to determine the effect of AM water extract on insulin activity, the GTT was carried out on all three groups of mice i.e., control, diabetic and treated according the method described earlier with slight modifications (Gupta *et al*., 2005). GTT was performed by oral administration of glucose load of 1 g kg^{-1} b.wt. in 0.1 mL of water to over night fasted animals. Blood samples were collected form the tail vein at 30, 60, 90, 120 min after the oral glucose load and treated as before for plasma glucose analysis.

Estimation of lipid profile in blood sample: After completion of the treatment, blood samples were collected and lipid profile for all the three groups of animals were performed using commercially available kits. Total cholesterol, High-Density Lipoprotein (HDL) cholesterol

and triglyceride (TG) levels in serum were determined according to the instruction of the manufacturer (Transasia Bio Medical Ltd., Mumbai, India). For the determination of Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) cholesterol Friedewald's formula (Friedewald *et al*., 1972) was used which states: LDL cholesterol = Triglyceride/5 and LDL cholesterol = Total Cholesterol-(VLDL+ HDL cholesterol).

Biochemical estimation of enzyme activities and tissue glycogen content

Glucose 6 phosphatase in liver: Glucose-6-phosphatase catalyzes the conversion of glucose-6-phosphate to glucose. This enzyme activity was determined as per the method described earlier with slight modifications (Baginsky *et al*., 1974). Briefly, the mice from the entire three groups were sacrificed and the collected liver was homogenized in ice cold sucrose solution (250 mM). To 0.1 mL of sucrose/EDTA buffer, 0.1 mL of 100 mM glucose-6-phosphate, 0.1 mL of imidazole buffer (100 mM, pH 6.5) and 0.1 mL of liver homogenate were added with thorough mixing. The tubes were than incubated at 37°C for 15 min. The reaction was terminated by the addition of 2 mL of TCA/Ascorbate (10:3% w/v) and the solution was centrifuged at 3000 rpm for 10 min. To 1 mL of clear supernatant 0.5 mL of ammonium molybdate (1% w/v) and 1 mL of sodium citrate (2% w/v) were added and the absorbance was measured at 700 nm. The enzyme activity was expressed as unit per gram tissue.

Hexokinase activity in liver: The hexokinase activity was tested based on the reduction of NAD through a coupled reaction with glucose 6 phosphate dehydrogenase as per the method described earlier (Brandstrup *et al*., 1957). The absorbance for reduced NAD was measured at 340 nm.

Tissue level of glycogen content: Glycogen content of liver and skeleton muscle was measured according to earlier established method (Sadasivam and Manickam, 1996; Maiti *et al*., 2004). Samples were homogenized separately in warm 80% ethanol at the concentration of 100 mg mL^{-1} and than centrifuged at 10,000 rpm for 20 min. The residue was collected and allowed to dry over a water bath. To each residue 5 mL of distilled water and 6 mL of perchloric acid was added. The extraction was further done at 4°C for 20 min. The collected extract was centrifuged at 10,000 rpm for 15 min and the supernatant was collected. From the supernatant, 0.2 mL was transferred to a graduated test tube and the volume was increased to 1 mL by addition of distilled water. Similarly a graded glycogen standard was made with a volume of 1 mL. To each tube added 4 mL of anthrone reagent and incubated at 95°C in a boiling water bath for 10 min. The absorbances of the samples were measured at 630 nm after cooling the tube at room temperature. The amount of glycogen in tissue samples was expressed in microgram of glucose per milligram tissue.

Analyzing the mechanism of action of plant extract as anti-diabetic agent

Induction of insulin release from pancreatic islet cell: Isolation of pancreatic islet cells was made according to the method reported earlier (Xia and Laychok, 1993; Gupta *et al*., 2005) with slight modification. After removing the pancreas form normal (group 1) and diabetic (group 2) mice, they were perfused with Hank's Balanced Salt Solution (HBSS) (pH 7.4) for about 15 min to remove blood and endogenous insulin. This was followed by finely mincing the tissue and then they were incubated for 30 min at 37°C with rapid magnetic stirring in a solution of crude collagenase (4 mg mL^{-1}) in HBSS containing 0.3% glucose and 1% Bovine Serum Albumin (BSA) pH 7.4. The next step, the separation of the islets from acinar tissues was done with ficoll (type-400) (Himedia, Mumbai, India) with varying gradients followed by centrifugation. The islet cells were picked up from the interphase of 11-20% gradient by Pasteur pipette. The purity of the islet cells were checked by Gomori chromium hematoxylin phloxin stain (Gomori, 1941). After dividing 30 islets per batch they were preincubated with glucose-Krebs Ringer Bicarbonate (KRB) buffer along with $NaHCO_3$ 0.2%, HEPES 0.38%, insulin-free BSA 0.1% and glucose 11.1 mM, for 5 min at 37°C in CO_2 incubator. The induction was continued for another 1 h after adding various concentrations of plant extracts (25, 50 and 100 μg) or buffer for controls. Aliquots of 50 μL were removed from the incubation mixture at the end of the incubation (i.e., 1 h) and were stored at -20°C till insulin assay. Insulin was measured using commercially available ELISA kits.

Estimation of glucose uptake by mouse psoas muscle tissue: Psoas muscles, immediately after isolation were placed in a KRB buffer (containing 11.1 mM glucose). The muscle tissue after isolation were processed and incubated following the method described by Gupta *et al*. (2005). The tissues were cut into pieces of about equal mass (100 mg) and preincubated for 5 min in CO_2 incubator as mentioned above. The tissues were then incubated with or without insulin (25 μg) in the presence of 100 μg of aqueous leaf extract for 150 min. Aliquots of 25 μL were removed from the incubation mixture at various time points and glucose concentration was determined.

Statistical analysis: The statistical significance was evaluated by one-way ANOVA using 5% level of significance followed by multiple two-tailed student's t-test (Sokal and Rohle, 1997). The statistical package used was SPSS (Ver. 10, SPSS Inc., USA).

RESULTS

Body weight: The body weight was found to drop significantly in streptozotocin induced diabetic mice in comparison to its control counterpart (non-diabetic) ($p<0.05$). However, after the administration of the leaf extract, the weight of the animal recovered to almost to the normal level (Table 1).

Fasting blood glucose level and GTT: Blood glucose estimated in 16 h fasting diabetic mice were significantly elevated. However this level was reduced down to close that of control mice upon treatment with 300 mg kg^{-1} b.wt. of the plant extract (FBG data, Table 2).

For GTT, overnight fasted mice were fed with 1 g kg^{-1} b.wt. of glucose and the blood glucose level was determined up to 120 min. The blood glucose level came down almost close to control (non-diabetic mice) by 90 min and this level was maintained until 120 min with an effective dose of 300 mg kg^{-1} b.wt. of extract (Table 2).

Estimation of lipid profile: Various parameters of blood lipid profiles were tested in streptozotocin induced diabetic mice before and after the treatment of the plant extract. The levels of TC, LDL, VLDL cholesterol and TG in diabetic mice were significantly less in the plant extract treated animals ($p<0.05$). About 40, 61 and 22% fall in TC, LDL cholesterol and TG level, respectively was found in diabetic mice treated with plant extract. In addition, HDL levels were found to be increases by 20% (as compared to diabetic control) in diabetic mice after treatment with AM leaf extract (Table 3).

Glucose-6-phosphatase activity in liver: After 15 days of treatment with aqueous extract of AM plant leaves, there was a significant reduction in liver glucose-6-phosphatase activity in the treated group compared to the untreated streptozotocin induced diabetic mice. The reduced level of enzyme activity (by 35%) was almost same as that of the non-diabetic control mice (Fig. 1).

Hepatic hexokinase activity: As compared to non diabetic control, the mean value of hepatic hexokinase activity decreased in diabetic mice (Fig. 2). The respective percentage decrease was almost 40% in diabetic

Table 1: Effect of aqueous leaf extract of *Aegle marmelos* on body weight in streptozotocin induced diabetic male mice

Groups	Initial body weight (g)	Final body weight (g)
Control	35±3.5	37±2.7
Diabetic	37±2.9	29±2.9
Treated	37±3.2	35±2.5*

n = 5 in each group, value are mean±SEM, *: Indicates significant difference with respect to diabetic control ($p<0.05$)

Table 2: Effect of plasma glucose level after the administration of *Aegle marmelos* leaf extract in streptozotocin induced diabetic male mice

	Plasma glucose level (mg dL^{-1})				
		30	60	90	120
Groups	FBG	(min)			
Diabetic	210.4±12.3	263.0±12.3	226.6±10	219.1±13.7	209.8±10.6
Control	92.6±6.7	145.8±11.6	130.2±9.1	120.2±8.0	99.0±6.8
Treated	135.2±6.4*	182.2±3.6*	160.6±8.1*	148.4±8.0*	119.6±5.9*

n = 5 in each group, value are mean±SEM, *: Represents statistically significant as compared to diabetic group ($p<0.05$)

Table 3: Plasma level of different lipids after the administration of *Aegle marmelos* leaf extract in streptozotocin induced diabetic male mice

	Plasma lipid level (mg dL^{-1})				
Treatment group	TC	HDLC	LDLC	TG	VLDLC
Control	98±3.3	23.0±3.7	57.0±4.3	88±3.6	17.6±3.4
Diabetic	187±4.5	19.4±4.1	143.0±2.3	121±3.5	24.2±2.9
Diabetic+AM	114±2.7*	38.0±2.3*	57.2±3.9*	94±3.7*	18.8±3.5*

Each value represents the mean±SEM, *: Represents statistically significant as compared to diabetic group ($p<0.05$), AM: *Aegle marmelos*

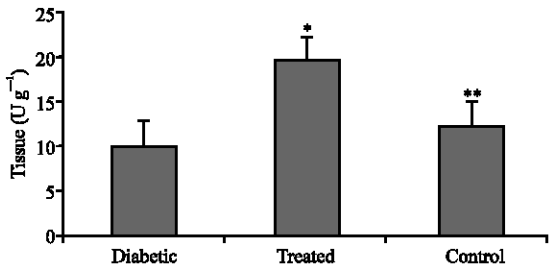

Fig. 1: Effect of aqueous leaf extract of *Aegle marmelos* on hepatic glucose-6-phosphatase activity in streptozotocin induced diabetic male mice. Data are expressed as mean±SEM; n = 5. * and ** indicates the significant level of differences in enzyme levels as compared to non-diabetic control and untreated diabetic control, respectively ($p<0.05$)

controls. Treatment of the animal with the AM leaf extract (300 mg kg^{-1} b.wt.) led to a rise in activity of this enzyme by 50% ($p<0.05$), as compared to diabetic control.

Glycogen level in tissue: Glycogen content of liver and skeletal muscles were estimated on 15 day of the treatment with AM plant extract in non-diabetic control, diabetic and diabetic treated mice as shown in Fig. 3. In diabetic mice both liver and muscle glycogen content fell significantly by 33 and 31%, respectively as compared to

Table 4: Effect of water extract on glucose uptake from medium by psoas muscle isolated from mouse

Treatment groups	Glucose uptake (mg dL^{-1}) 30 (min)	60	90	120	150
MT	18±6.2	27±4.5	32±1.4	34±4.3	42±3.1
MT + E	22±5.9*(22)[§]	29±4.4*(7.4)[§]	35±3.6*(9)[§]	39±4.3*(14.7)[§]	43±1.5*(2.3)[§]
MT + I	35±2.9	47±3.8	53±3.3	65±2.9	74±4.1
MT + I + E	43±5.9*(23)[γ]	59±6.9*(26)[γ]	68±3.6*(28)[γ]	75±3.7*(15.4)[γ]	84±4.2*(13.5)[γ]

Values are mean±SEM of three independent experiments each performed in quadruplicates. MT: Muscle tissue; E: Extract; I: Insulin, Extract and insulin in the incubation were added at a concentration of 100 and 25 μg, respectively. [§]: Value in the bracket indicates percentage increase when compared with muscle tissue alone at the particular time point. [γ]: Values in the bracket indicate percentage increase when compared with muscle tissue with extract at that particular time point. * $p<0.05$ when compared to control (only MT)

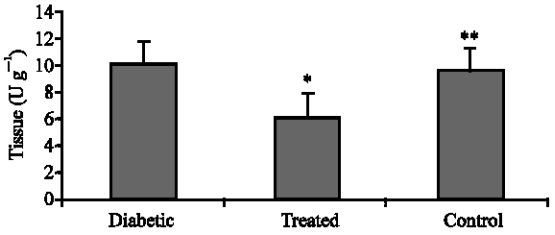

Fig. 2: Effect of aqueous leaf extract of *Aegle marmelos* on hepatic hexokinase activity in streptozotocin induced diabetic male mice. Data are expressed as mean±SEM; n = 5. * and ** indicates the significant level of differences in enzyme levels as compared to non-diabetic control and untreated diabetic control, respectively ($p<0.05$)

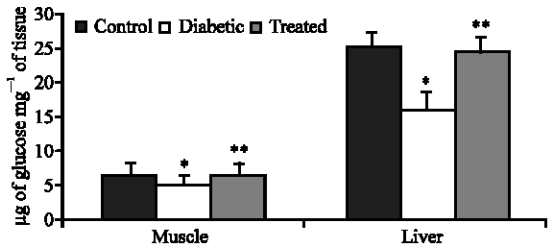

Fig. 3: Effect of aqueous leaf extract of *Aegle marmelos* on liver and muscle glycogen contents in streptozotocin induced diabetic male mice. Data are expressed as mean±SEM; n = 5. * and ** indicates the significant level of difference in glycogen levels as compared to control (non-diabetic) and untreated diabetic control, respectively ($p<0.05$)

non-diabetic control ($p<0.05$). Treatment with AM extract led to a 41 and 40% increase respectively in hepatic and muscle glycogen content in diabetic group ($p<0.05$ as compared to diabetic control).

Release of insulin from pancreatic islets: The release of insulin from pancreatic islet cells was determined from

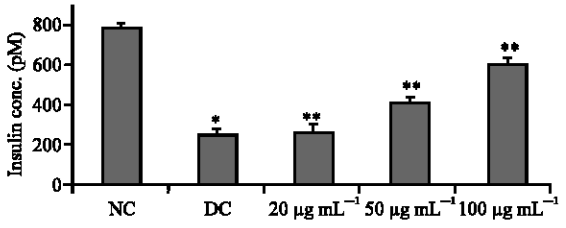

Fig. 4: Levels of insulin released in the cell culture media on treatment of mice pancreatic islet cells with various concentration of *Aegle marmelos* leaf extract. Data are expressed as mean±SEM of four separate experiments each performed in quadruplicates. * Indicates statistically significant as compared to control and ** indicates significant as compared to untreated diabetic control ($p<0.05$). NC, Normal control; DC, Diabetic control

diabetic mice with increasing concentration of AM plant extract. Figure 4 shows that the isolated islet cells on treatment with increasing concentration of plant extract (25, 50 and 100 μg) for 1 h, resulted in a linear rise in insulin release into the medium and at highest concentration of extract added (100 μg) it is about 3 folds as compared to that of the diabetic control. Albeit it could not reach the level as that of the normal mice, yet it justifies the role of this plant extract in induction of insulin release from the pancreatic islets, the mechanism of which is yet to be determined.

Glucose uptake by the muscle tissue: Uptake of glucose by psoas muscle of mice by aqueous extract of AM leaves in the presence and absence of insulin was studied by measuring the decrease in glucose concentration in the incubation medium with time. On treatment of the muscle tissues with plant extract there was a gradual decrease in glucose level in the media from 30 to 150 min incubation (indicated as increase in cellular concentration of glucose by transfer of glucose from media to cells) (Table 4). It is evident from these results that 100 μg of the water extract

alone by itself increased glucose uptake by about 22% up to 30 min, after which the effect gradually decreased by another 120 min. However, in the presence of insulin, increase in glucose uptake was 28% by 90 min and it decreased to 13.5% in 150 min. As per the data, the presence of insulin stabilizes the rate of glucose uptake by muscle tissue in the presence of AM plant extract which is the indicative fact of the synergetic activity of insulin and AM leaf extract.

DISCUSSION

Diabetes is a chronic metabolic disorder affecting a major population worldwide. A sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications (Kim *et al*., 2006). The conventional therapies for diabetes have many shortcomings like unwanted side effects and high rate of secondary failure. On the other hand, herbal extracts are expected to have similar efficacy without side effects like that of conventional drugs. The present investigation reports the anti-diabetogenic and hypoglycemic effects of aqueous extract of leaves of *Aegle marmelos* on streptozotocin-induced diabetic mice. Streptozotocin is a glucoseamine-nitrosourea compound used in medicinal research to produce animal models for diabetes mellitus. As with other alkylating agents in the nitrosourea class, it is toxic to cell by causing damage to the DNA, though other mechanisms may also contribute. Thus streptozotocin injection results in diabetes mellitus due to the destruction of β-cells of islets of Langerhans as proposed by many authors (Bell and Hye, 1983; Ozturk *et al*., 1996). This effect is evident by high level of glucose in animals. The data presented here could provide a base for understanding the exact molecular mechamism of action of this plant's active principles.

Loss of body weight, weakness, polyuria and polyphagia are some of the symptoms associated with type 1 diabetes mellitus (Maiti *et al*., 2004). Weight loss was found very distinctly in our streptozotocin induced diabetic mice. After treatment of mice for 15 days with AM plant leaf extracts, the mice regained their weight, which was close to the control (non diabetic mice) level. This was further confirmed by the alteration in the fasting blood glucose levels in diabetic mice followed by its regeneration after the plant extract treatment. However there was no significant alteration in fasting blood glucose in the control mice. Further, this same extract resulted in the significant reduction of peak level of sugar within 2 h time and this fact strengthens the anti-diabetogenic potentiality of this plant extract as reported by many authors earlier in rat models (Karunanayake *et al*., 1984; Grover *et al*., 2002). The plant extract might enhance glucose utilization since it significantly decreased the blood glucose level in glucose-loaded mice (GTT). This fact could be attributed to the potentiation of insulin effect of plasma by increasing the pancreatic secretion of insulin from existingβ-cell or its release from bound insulin. In this context, a number of other plants have been observed to have hypoglycemic effects (Kasiviswanath *et al*., 2005; Eidi *et al*., 2006).

The administrations of AM extract significantly decreased serum triglycerides and total cholesterol in diabetic mice. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents the high risk of coronary heart diseases (Davidson, 1981). The marked hyperlipidemia that characterizes the diabetes status may be regarded as consequences of the uninhibited action of lipolytic hormones on the fat depots (Davis, 2006). Lowering of serum lipid concentration through dietary or drug therapy seems to be associated with a decrease in the risk of vascular diseases (Rhoads *et al*., 1976; Kesari *et al*., 2006). In consistence with the present data other workers have reported that the administration of AM plant leaf and seed extracts to alloxan induced diabetic rats improved the serum cholesterol levels as compared to control and this effect was also similar to insulin treatment (Ponnachan *et al*., 1993b; Kesari *et al*., 2006). With respect to cholesterol lowering property of AM leaf extract, it could be suggested that the constituents of the plant extract, may act as inhibitors for enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in *de novo* cholesterol biosynthesis as has been suggested for some plants earlier (Gebhardt and Beck, 1996; Eidi *et al*., 2006). Consistent with this idea Sabu and Kuttan (2004) reported that the *in vivo* treatment of AM leaf extract to alloxan induced diabetic rats reduces the lipid peroxidation product which further supports our findings.

Glycogen is the primary intracellular storage form of glucose and its levels in various tissues, specifically in liver and skeletal muscles, are a direct reflection of insulin activity since it regulates glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since streptozotocin causes selective destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it could be predicted that glycogen levels in tissues (muscle and liver) decreases as the influx of glucose in the liver is inhibited in the absence of insulin (Saltiel and Khan, 2001). However, this alteration in hepatic and muscle glycogen content is normalized by insulin treatment (Weber *et al*.,

1966; Vats *et al*., 2004). Our results showed that supplementation of diabetic mice with AM extract resulted in significant elevation in both muscle and hepatic glycogen content. This point towards one of the possible ways the plant extract might act by improving the glycogenesis process in muscle and liver.

Decreases in the activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animals resulting in depletion of liver and muscle glycogen content (Grover *et al*., 2000). In order to understand the biochemical mechanism of action of the AM leaf extract as anti-diabetogenic agent, the study was done to estimate the level of hepatic hexokinase in diabetic mice before and after treatment with AM plant extract. The present study shows the similar pattern of reduced hexokinase level in streptozotocin treated diabetic mice. Treatment with AM plant extract increased the level of enzyme to the control level, indicating an over-all increase in glucose influx. Similarly, glucose-6-phosphatase, has also been reported to play an important role in glucose homeostasis in liver and kidney by activating the gluconeogenic pathway (Berg *et al*., 2001). In this study, the glucose-6-phosphatase activity in the liver was elevated in streptozotocin induced diabetic mice and this was consistent with earlier reports in diabetic animals and human models (Gupta *et al*., 1999). The AM leaf extract also resulted in significant reduction of the enzyme activity close to control level thus making way for proposing another mode of anti-diabetic activity of this plant. This result is similar to that previously reported where several potential herbal plant extract has been known to improve the diabetic condition (Gupta *et al*., 1999).

In an attempt of further understand the mechanism of action of AM plant extract at *in vitro* cellular level, the pancreatic islet cells were isolated from mice and treated with different concentrations of extracts. Results showed that the water extract caused a significant release of insulin from pancreatic cells. Even though the release of insulin from the pancreatic tissues is small, it is indicative of the probable mode of action of this plant extract. This supports our earlier unpublished finding that the increase in serum level of insulin in extract treated diabetic mice is due to direct action of the extract on pancreatic β-cells. A similar pattern in experiment and result has also been reported recently by Gupta *et al*. (2005) using another plant extract.

In type II diabetes, more often the cause is the lack of insulin sensitivity or insulin resistance at the receptor or post receptor level, rather than lack of insulin. To gain an insight into this aspect if AM might involve, our studies on psoas muscle indicated that the water extract resulted in the uptake of glucose from the medium within 30 min of the incubation. This effect was further enhanced in the presence of insulin. This result reflects two possibilities: either it has insulin like effect on psoas muscle or direct stimulatory effect on the enzymes involved in the metabolism of glucose. According to some authors (Gupta *et al*., 2005), increase of glucose uptake in the presence of insulin suggests the possibility of increased binding of insulin to receptors in the muscle or increase in the number of insulin receptor. Whatever the reason could be this particular aspect of the AM leaf extract action needs to be studied in detailed before proposing any hypothesis on its mode of action.

From the above data it could be conceived that the aqueous extract of AM plant may contain some biomolecules that may sensitize the insulin receptor to insulin or stimulate the β-cells of islets of Langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level. To understand the exact molecular mechanism of action of this plant extract, study is in progress to identify, isolate and purify the bioactive molecules from this plant extract.

In conclusion, the data obtained from the present study indicates that the *Aegle marmelos* leaf extract could have some bioactive molecules, which may have beneficial effects as hypoglycemic, anti-hyperglycemic and hypolipidemic agents. The exact mechanism of action needs further investigation. However the present study gives some preliminary idea that the AM leaf extract has the potential to act at multiple sites. Toxicity data has already proven that the dose used in this investigation is far below the LD_{50} of the extract and did not show any change in the blood parameters as in shown in case of toxicity (data not shown). Further studies on possible usefulness of AM leaf extract in the treatment of diabetes mellitus are encouraged.

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REFERENCES

Alam, M.M., M.B. Siddiqui and W. Husain, 1990. Treatment of diabetes through herbal drug in rural India. Filoterapia, 61 (3): 240-242.

Baginsky, E.S., P.P. Foa and B. Zad, 1974. Glucose 6-phosphatase. In: Bergmeyer's Methods in Enzymatic Analysis, Vol. 2. Academic Press, New York, pp: 788-792.

Bell, R.H. Jr. and R.J. Hye, 1983. Animal models of diabetes mellitus: Physiology and pathology. J. Surg. Res., 35 (5): 433-460.

Berg, J.M., J.L. Tymoczko and L. Stryer, 2001. Glycolysis and Gluconeogenesis. In: Biochemistry, Berg, J.M., J.L. Tymoczko and L. Stryer (Eds.). W.H. Freeman and Company, New York, pp: 425-464.

Brandstrup, N., J.E. Kirk and C. Bruni, 1957. Determination of hexokinase in tissues. J. Gerentol., 12: 166-171.

Chakrabarti, B., C. Mallick and S. Bhattacharya, 1960. Studies on the green leaves of *Aegle marmelos* and *Piper nigrum* on the glucose and cholesterol levels of blood in diabetes mellitus. Indian Med. Forum., 9: 285-286.

Das, A.V., P.S. Padyatti and C.S. Paulsoe, 1996. Effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. on histological and ultra structural changes in tissues of streptozotocin induced diabetic rats. Indian J. Exp. Biol., 34 (4): 341-345.

Davidson, M.B., 1981. Diabetes Mellitus Diagnosis and Treatment. Wiley, New York, pp: 27, 48, 109, 157.

Davis, S.N., 2006. Insulin, Oral Hypoglycemic Agents and the Pharmacology of the Endocrine Pancreas. In: Godman and Gilman's. The Pharmacological Basis of the Therapeutics, Brunton, L.L., J.S. Lazo and K.L. Parker (Eds.). McGraw-Hill, New York, pp: 1613-1645.

Dubey, G.P., S.P. Dixit and S. Alok, 1994. Alloxan-induced diabetes in rabbits and effect of herbal formulation D-400. Indian J. Pharmacol., 26 (3): 225-226.

Eidi, A., M. Eidi and E. Esmaeili, 2006. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine, 13 (9-10): 624-629.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Fuller, J.H., M.J. Shipley, G.J. Rose, R.J. Jarrett and H. Keen, 1980. Coronary heart disease risk and impaired glucose tolerance. The Whitehall Study. Lancet, 1 (8183): 1373-1376.

Gebhardt, R. and H. Beck, 1996. Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids, 31 (12): 1269-1276.

Gomori, G., 1941. Observation with differential stains on human islet of Langerhans. Am. J. Pathol., 17 (3): 395-406.

Grover, J.K., V. Vats and S.S. Rathi, 2000. Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cardifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J. Ethnopharmacol., 73 (3): 461-470.

Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol., 81 (1): 81-100.

Gupta, D., J. Raku and N.Z. Baquer, 1999. Modulation of some gluconeogenic enzyme activities in diabetic rat liver and kidney: Effect of antidiabetic compounds. Indian J. Exp. Biol., 37 (2): 196-199.

Gupta, R.K., A.N. Kesari, G. Watal, P.S. Murthy, R. Chandra, K. Maithal and V. Tandon, 2005. Hypoglycemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animal. Curr. Sci., 88 (1): 1244-1254.

Kamalakkannan, N. and P.S.M. Prince, 2003. Hypoglycemic effect of water extract of *Aegle marmelos* fruits in streptozotocin diabetic rats. J. Ethnopharmacol., 87 (2-3): 207-210.

Kamalakkannan, N. and P.S.M. Prince, 2004. Antidiabetic and antioxidant activity of *Aegle marmelos* extract in streptozotocin induced diabetic rats. Pharma. Biol., 11 (2): 223-231.

Karunanayake, E.H., J. Welihinda, S.R. Sirimanne and G. Sinnadori, 1984. Oral hypoglycemic activity of some medicinal plants of Sri Lanka. J. Ethnopharmacol., 11 (2): 223-231.

Kasiviswanath, R., A. Ramesh and K.E. Kumar, 2005. Hypoglycemic and antihyperglycemic effect of *Gmelina asaistica* Linn. In normal and alloxan induced diabetic rats. Biol. Pharm. Bull., 28 (4): 729-732.

Kesari, A.N., R.K. Gupta, S.K. Singh, S. Diwakar and G. Watal, 2006. Hypoglycemic and hyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. J. Ethnopharmacol., 107 (3): 374-379.

Khare, C.P., 2004. *Aegle marmelos*. In: Indian Herbal Remedies, Khare, C.P. (Ed.). Springer, USA, pp: 27-29.

Kim, S.H., S.H. Hyun and S.Y. Choung, 2006. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J. Ethnopharmacol., 104 (1-2): 119-123.

Ladeji, O., I. Omekarah and M. Solomon, 2003. Hypoglycemic properties of aqueous extract of *Ceiba pentandra* in streptozotocin-induced diabetic rats. J. Ethnopharmacol., 84 (2-3): 241-245.

Maiti, R., D. Jana, U.K. Das and D. Ghosh, 2004. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. J. Ethnopharmacol., 92 (1): 85-91.

Mukherjee, P.K., K. Maiti, K. Mukherjee and P.J. Houghton, 2006. Leads from Indian medicinal plants with hypoglycemic potentials. J. Ethnopharmacol., 106 (1): 1-28.

Ozturk, Y., V.M. Altan and A.N. Yildizoglu, 1996. Effects of experimental diabetes and insulin on smooth muscle functions. Pharmacol. Rev., 48: 69-112.

Ponnachan, P.T.C., C.S. Paulose and K.R. Panikkar, 1993a. Hypoglycemic effect of alkaloid preparation from leaves of *Aegle marmelos*. Amla Res. Bull., 13: 37-41.

Ponnachan, P.T.C., C.S. Paulose and K.R. Panikkar, 1993b. Effect of leaf extract of *Aegle marmelos* in diabetic rats. Indian J. Exp. Biol., 31 (4): 345-347.

Prakash, V., 1992. Diabetes management-some useful herbs. Jeevamiya Shishir, pp: 10.

Prince, P.S., V.P. Menon and L. Pari, 1998. Hypoglycemic activity of *Syzigium cuminii* seeds: Effect on lipid peroxidation in alloxan diabetic rats. J. Ethnopharmacol., 61: 1-7.

Rao, B.K., P.R. Sudarshan, M.D. Rajasekhar, N. Nagaraju and C.A. Rao, 2003. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. J. Ethnopharmacol., 85 (1): 169-172.

Rhoads, G.G., C.L. Gulbrandse and A. Kagan, 1976. Serum lipoprotein and coronary artery disease in a population study of Hawaiian Japanese men. New England J. Med., 294 (56): 293-298.

Sabu, M.C. and R. Kuttan, 2004. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian J. Physiol. Pharmacol., 48 (1): 81-88.

Sachdewa, A., D. Raina, A.K. Srivastava and L.D. Khemani, 2001. Effect of *Aegle marmelos* and *Hibiscus rosa sinensis* leaf extract on glucose tolerance in glucose induced hyperglycemic rats (Charles foster). J. Environ. Biol., 22 (1): 53-57.

Sadasivam, S. and A. Manickam, 1996. Carbohydrates. In: Methods in Biochemistry, Sadasivam, S. and A. Manickam (Eds.). New Age International Pvt. Ltd., New Delhi, pp: 11-12.

Saltiel, A.R. and C.R. Khan, 2001. Insulin signaling and the regulation of glucose and lipid metabolism. Nature, 414: 799-806.

Sanae, F., K.I. Miyamoto, H. Sawanishi, H. Kizu, Y. Tomimori, J.N. Sun and X.H. Sun, 1998. Hypoglycemic effect of Shokatsu-Cha (Xiao-Ke-Ca) in streptozotocin induced diabetes mellitus. Phytother. Res., 10 (2): 127-130.

Seema, P.V., B. Sudha, P.S. Padayatti, A. Abrahm, K.G. Raghu and C.S. Paulsoe, 1996. Kinetic studies of purified malate dehydrogenase in liver of streptozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. Indian J. Exp. Biol., 34 (6): 600-602.

Sokal, R.R. and F.J. Rohle, 1997. Introduction to Analysis of Variance. In: Biometry, Sokal, R.R. and F.J. Rohle (Eds.). W.H. Freeman and Company, New York, pp: 179-206.

Upadhya, S., K.K. Shanbhag, G. Suneetha, N.M. Balachandra and S. Upadhya, 2004. A study on hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. Indian J. Physiol. Pharmacol., 48 (4): 476-480.

Vats, V., S.P. Yadav and J.K. Grover, 2004. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. J. Ethnopharmacol., 90 (1): 155-160.

Weber, G., M.A. Lea and E.A. Fisher, 1966. Regulatory pattern of liver carbohydrate metabolizing enzymes: Insulin as an inducer of key glycolytic enzymes. Enzymol. Biol. Clin. (Basel)., 7 (1): 11-24.

Xia, M. and S.C. Laychok, 1993. Insulin secretion myoinositol transport and Na^+-K ATPase in glucose desensitized rat islets. Diabetes, 42 (10): 1392-1400.