Anti-hyperglycemic activity of alcoholic leaf extract of *Aegle marmelos* (linn.) on alloxan induced diabetic rats

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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 a one of the best Ayurveda medicine for Diabetes Mellitus. The present study was performed to evaluate the hyperglycemic effect of aqueous extract of *A. marmelos* leaves on diabetic rats. An ethanolic extract of *A. marmelos* was found to be reducing the blood sugar in alloxan induced diabetic rats. Reduction in blood sugar could be seen from 7th day after continuous administration of the extract and on 28th 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 *A. marmelos* extract. The ethanolic extract of *A. marmelos* leaves have a promising antidiabetic activity against alloxan – induced diabetic rats.

Keywords: *Aegle marmelos*, Alloxan, Antidiabetic, Hyperglycemia.

1. Introduction

Diabetes Mellitus is the one of the challenging health problem in twenty-first century that is occurring throughout the world today. It is a familiar debilitating disorder [1]. It is a syndrome of disordered metabolism characterized by hyperglycemia due either to an unlimited lack of insulin secretion or insulin action or both. The metabolic dysregulation associated with DM inflicts widespread injure on multiple organs. This imposes a incredible burden on diabetic patients. It will keep on being a most important cause of morbidity and mortality in prospect [2].

Insulin is the most important hormone controlling the intermediary metabolism of our body. Its overall effect is to keeps energy by facilitating the update and storage of glucose, amino acids and fats after meals. Acutely, it reduces the sugar level in blood. Consequently, a plunge in plasma insulin increases sugar level in the blood. Insulin is the important and first protein for which an amino acid sequence was determined. It has two peptide chains such as A and B, of 21 and 30 amino acid residues, respectively. Insulin is synthesized in β-cells of pancreas as a single chain 86-amino acid precursor polypeptide (preproinsulin) in the rough endoplasmic reticulum. The main thing controlling the synthesis and secretion of insulin is the concentration of glucose in blood. There is a stable basal release of insulin and also response to adjust in blood glucose. Insulin plays an important role in the metabolism of carbohydrate, fat and protein [3].

Bael (*Aegle marmelos*) is one of the traditional and an imperative medicinal plant. It is the family of Rutacae, is also known as Bale fruit tree, and is a restrained sized, slender, aromatic tree. The various bio-chemicals present in *A. marmelos* leaves are alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids [4, 5]. Apart from leaves the fruits of the plant also having many of the phyto-chemicals such as carbohydrates, protein, fiber, fat, calcium, phosphorus, potassium, Iron, minerals and vitamins (Vitamin A, Vitamin B1, Vitamin C and Riboflavin), steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat and oil, inulin, proteins, alkaloids, cardiac glycosides and flavonoids [6].

A. marmelos having lot of medicinal properties such as antibacterial agent against *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Proteus vulgaris*, *Staphylococcus aureus*, *B. subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *S. paratyphi* A, *S. paratyphi* B, *Micrococcus luteus*, *Enterococcus faecalis* and *Streptococcus faecalis* [7-11]. And also this plant has excellent antifungal activity against *Penicillium chrysogenum*, *Fusarium oxysporum*, *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M. gypseum*, *Epidermophyton floccosum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans* [7, 9, 12]. Apart from this plant having various medicinal properties were shown in Table 1.

Diabetes mellitus is a chronic, widely spread human disease, experimental induction of diabetes mellitus in animal models in essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to

induce diabetes mellitus in laboratory animals with variable success and many difficulties. Animal models in research work find advantage especially on diabetes, where various aspects of the disease like the etiology, it multifactorial genetics, pathogenesis of the disease and the complications are explicitly understood. Induction of diabetes in animals can be carried by using different chemical diabetogens are surgically by partial pancreatectomy, viral induction and genetic manipulation by selective inbreeding. Among all the above methods of experimental diabetes, chemical diabetogens such as Alloxan and Steptozotocin are more widely used.

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type l diabetes in human. Alloxan is selectively toxic to insulin- producing pancreatic beta cells because it preferentially accumulates i n beta cells through uptake via the GLUT2 glucose transporter.

2. Materials and methods

2.1 Animals

Rats used in this experiment were highly inbred male Wister Albino rats from laboratory (APCAS). The rat weighed 160g were used. The animals were housed in special caged under hygienic conditions (13h light 12h dark cycle at room temperature) and maintained on commercial pellet diet containing protein – 21% lipid -5% nitrogen free extract -55% and provided with metabolically energy at 3600Kcal/Kg and also enriched by vitamins and minerals. It was supplied by the "Hindustan Lever Limited" Mumbai marked under the trade name "Gold Mohur Feeds" water was provided. The rats were kept in animal house for ten days before starting the experiments. Ethical clearance was obtained from the Institutional Animal Ethical Committee, CPCSEA, India (Reg No.282/ac/09/CPCSEA).

2.2 Induction of diabetes mellitus

Alloxan monohydrates induced diabetes mellitus was produced in a batch of hypogenic male albino rats by injecting intrapreophrea; cavity a single dose (40mg/Kg weight) of 2% alloxan monohydrates solution in saline, after these have been fasted for 24hours. This single dose of alloxan produced persistence hyperglycemia after 7 days it was observed that the condition for 5 days. The animals showed the following signs of the condition: Polydipsia (abnormal thirst), Polyuria (increased urine volume), weight loss (due to lean mass loss), asthenia weakness (due to the inability to use glucose as a source of energy), dehydration (due to the animal body's attempts to get rid of the excess blood glucose as the normal process of storing glucose in the body cells in impair.

In order to assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done in any of the four veins in the tail of the rat 7 days after induction. After 7 days start (0r) begin the treatment to the rats [21-24].

2.3 Preparation of plant material: leaves

Aegle marmelos (leaves) was collected from the local temple at Walajapet, Vellore District. The Collected leaves was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grinded into powder.

The leaves were dried under shade and coarsely powdered. The powder was successively extracted using soxhlet apparatus with ethanol and water. These extracts were condensed using rotary vacuum evaporator followed by vacuum evaporator and stored in desiccators. The powder of all the extracts was suspended in appropriate solvent systems. This extract was diluted with water (1:10) and was administered orally to rats.

2.4 Experimental design

Experimental animals are divided into five treatment groups.

Group -I (Normal)

Six albino rats are maintained in normal condition.

Group –II (Diabetic Induced)

The rats were made diabetic by administration of 2% Alloxan monohydrate. The rats were fasted for 16 hours but had been allowed free access to water. Alloxan monohydrate was dissolved in sterile normal saline immediately before use and injected intraperinotical cavity in a dose 2% Alloxan monohydrate solution in saline. The single dose of alloxan produces persistence hyperglycemia after 24 hours and it was observed that the condition was maintained for 6 days.

Group –III (A. marmelos control)

The rats were in normal condition control rats also maintain same procedure control rats receiving 2

ml of *A. marmelos* extract per day orally administered for every 24 hours for 20 days.

Group –IV (Diabetic Treatment)

Alloxan diabetic rats receiving 2ml of *A. marmelos* extract per day orally administrated for every 24 hours for 20 days.

Group V (Glibenclamide treatment)

Diabetes rats were given with glibenclamide (600 µg/Kg body weight) in aqueous solution daily introgastric tube for 20 days. The animals were dosed through the incubation every day before any food was given. Food and water were provided the duration of treatment was 20 days. After the treatment period, the rats were sacrificed, and blood was drawn from ventricles and serum separated for various tests is collected. Liver was excised from each animal; the tissue was washed with ice cold saline and homogenized in Tris HCl buffer PH-7.5. The serum obtained was used immediately for the estimation of blood glucose, total serum protein, serum triglycerides, urea and insulin.

2.5 Statistical analysis

The different of biochemical parameters were measured using the statistical method of Analysis of Variance (ANOVA). Analysis of variance refers to the examination of differences among the samples. It is an extremely useful technique concerning research in biology. It is a statistical technique specially designed to test whether the means of more than the quantities population are equal. The statistical significance was assessed using one-way Analysis of Variance (ANOVA) using SPSS 12.0 version (SPSS, Cary, NC, USA) followed by Bonferroni's multiple comparison test (BMCT). The values are expressed as mean ± SD and $p < 0.05$ was considered to be significant.

3. Results and Discussion

Diabetic is a group of metabolic disease characterized by hyperglycemia high blood sugar level. Non-insulin dependent diabetes mellitus is the commonest form of globerties as well as in India. Hereditary factor obesity sedentary life style and aging have been shown to raise the risk for diabetes. The proper medical care and a regular monitoring of diabetes are essential not only to keep the disease and the management. To prevent the varieties of other Diabetes related problems because no were cure has been identified. Hence, management of Diabetes with diet exercise and drug has been established.

Antidiabetic drugs treat diabetes mellitus by lowering blood glucose levels in the blood with the exceptions of insulin. All the drug administered orally, are also called "oral hypoglycemic agent", herbs for diabetes are used more and more to compliment or sometimes replace conventional diabetic drugs. It has been reported that Cinnamomum has insulin like activity and it contains like activity and it contains an active ingredient water soluble polyphenolic compound. It initiates insulin triggers in its receptor and work synergistically with insulin Cinnamomum also have anti lipidemic effect [2 5] .

The present study was conducted to find out the effect of oral administration of *A. marmelos* on normal and alloxan diabetic as approximation to the possible mechanism of action. Diabetes mellitus was induced in albino rats by injecting alloxan monohydrate into intraperitonial cavity a single dose of 40 mg/kg of body of 2% alloxan anhydrate solution in saline. After these, Rats have been kept fasting for 24 hours, hyperglycemia has been produced after one week. It was observed that a condition was maintained for 5 days. These animals were dosed through every day, before food and water provide for 20 days. After the treatment period the rats were sacrificed blood was drawn from ventricle and serum separated for various biochemical estimation.

The serum protein, Blood Glucose, serum triglycerides, urea, Insulin and liver tissue were used for the assay of enzyme activities of glutamate pyruvate transaminase (GPT). Overnight prescribed was then estimated to bring out positive conclusion the result are discussed with available data's describe below.

After the induction of diabetes by injecting freshly prepared alloxan through intraperitonial cavity, it was confirmed by testing of glucosuria in the urine using glucose indicator sticks, Diabetes induced within 7 days. The changes in the body weight of different experimental groups were noted.

The body weight of the alloxan induced diabetic group II rats was found to be reduced. On treatment with *A. marmelos* and Glibenclamide on Group IV and V, the body weight was gained comparing to the normal and control rats of Group I and III. These shows *A. marmelos* exhibited considerable gain of body weight. The value was increased in group II diabetic rats comparing on treatment with normal and control rat of group I and III *A. marmelos* and Glibenclamide the amount of food and volume was reverted back to the normal and treatment group of IV and V, increased fluid intake and food is the one of the symptoms of Diabetes which has been normalize on treatment effect of herbal.

Animal treated with alloxan induced diabetes group II shows a significant elevated in blood glucose when compared to group I and III of normal, control treated with *A. marmelos* extract. Alloxan, a β-islet cell cytotoxin, destroying the pancreatic β-cells leads to reduced secretion of insulin by the pancreatic islet cell [26].

A. marmelos treated group IV diabetic rats might enhance glucose utilization because of significantly reduces the blood glucose levels in treated rats (Table 2). This might be due to restoration of delays insulin response or inhibition of intestinal absorption of glucose due to reduction in the activity of intestinal glycosidases like sucrose, maltase and lactase in the small intestine. The similar reaction might carry out in group V treated with Glibenclamide. There was a marked reduction in the plasma protein content of untreated diabetic rats group II when compared to that of normal and control rats of group I and III. On administration of *A. marmelos* extract to diabetes rats restore the protein level almost equal to group V treated with Glibenclamide. It might be due to the increased uptake or glucose by the cell by stimulating the insulin receptor (IRS) I. This may inhibit the protein catabolism leads to positive nitrogen balance [27] (Table 2). The diabetic rat shows a significant decrease in plasma insulin. On treatment with an extract group IV restore the plasma insulin significantly compares to Glibenclamide treated Group V. The treatment extract to normal control rat of group III did not show significant effect of plasma insulin from the existing beta cells of pancreas (Table 2).

The level of urea and triglycerides which has increased after induction of diabetes was found to be decreased in group IV after treatment with *A. marmelos* extract almost equal to Glibenclamide treated group V rats. There are no any significant changes in the level of urea and triglycerides in the administration of *A. marmelos* of group III rats (Table 3).

4. Conclusion

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia- high blood sugar levels which results from defects in insulin secretion. The albino rats were induced diabetes by the intraperitonial injection of Alloxan. After the *A. marmelos* treatment of diabetic rats the blood glucose level returned back to near normal level. During diabetes condition the amount of food and volume of fluid intake is high when compared to the normal rats. The body weight is decreased in diabetic rats when compared to normal rats. After the *A. marmelos* treatment these levels were back to near normal level. Animal treated with alloxan induced diabetes shows a significant increase in blood glucose when compares to normal rats. Changes were observed in the levels of serum protein and insulin in diabetic condition. After the administration of *A. marmelos*, these levels are corrected to near normal level due to *A. marmelos* might be increase the release of insulin from the existing β –cells of pancreas. The levels of urea and Triglycerides were increased after induction of diabetes. After the administration of *A. marmelos* these levels were reverted back to near normal level. Overall, it may be concluded that *Aegle marmelos* extract possesses hypoglycemic potential and has been shown to afford significant protection against alloxan induced diabetes.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgements

The Authors would like to thank Dr.A.Mohamed sadiq, Principal, APCAS, Kalavai for his support and valuable suggestions.

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Figure Legends

Figure 1: *A. marmelos*

Table 1: Medicinal application of *A. marmelos*

Table 2: Levels of Blood glucose, Serum protein, and Plasma insulin in different group of rats

Table 3: Levels of Blood urea, Serum triglycerides in different groups of rats

Table 1

S. No	Applications	Reference
1	Antioxidant activity, DPPH radical scavenging method, reducing power assay, nitric oxide scavenging assay, superoxide radical scavenging assay, ABTS radical scavenging assay and H_2O_2 radical scavenging assay	[6]
2	Antibacterial activity	[7]
3	Antifungal activity	[12]
4	Antiproliferative effects against human tumor cell lines leukemic K562, T-lymphoid Jurkat, Blymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDAMB-231 cell lines	[13]
5	Analgesic activity	[14]
6	Anti-inflammatory activity	[15]
7	Antiarthritis activity	[16]
8	Hepatoprotective activity	[17]
9	Cytoprotective activity	[18]
10	Antidiarrheal Activity *Shigella boydii*, *S. sonnei* and *S. flexneri*, and *S. dysenteriae*	[19]
11	Larvicidal activity	[20]

Table 2

Parameter	Normal Group I	Inducer Group II	*A. Marmelos* Control Group III	*A. Marmelos* Treated Group IV	Glibenclamide Treated Group V
Glucose mg/dl	96.7±0.15	125.4±1.25	98.2±0.05	107.5±1.34	106.4±0.75
Protein g/dl	6.81±0.53	8.66±0.81	6.96±0.30	6.21±0.13	6.34±0.41
Insulin µ/ml	21.24±0.41	27.25±0.32	23.12±0.27	19.13±0.75	20.14±0.15

Values are mean ± S.D. for six individuals in each group P<0.05 values are considered statistically significant (BMCT)

Table 3

Parameter	Normal Group I	Inducer Group II	*A. Marmelos* Control Group III	*A. Marmelos* Treated Group Iv	Glibenclamide Treated Group V
Urea mg/dl	27.3±7.00	45.6±3.6	27.23±9.00	17.8±2.13	16.4±1.45
Triglycerides mg/dl	127.0±0.27	262.17±3.17	126.0±0.38	90.05±6.32	85.34±1.54

Values are mean ± S.D. for six individuals in each group P<0.05 values are considered statistically significant (BMCT)

Figure 1