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Biochemical evaluation of anti diabetic activity of aqueous extract of *Gmelina arborea* in Alloxan induced albino rats

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ABSTRACT
Diabetes mellitus is a clinical syndrome, a group of metabolic diseases which cells do not respond to the insulin that is produced. All forms of diabetes have been treatable since insulin became available in 1921. Though drugs are plenty for the treatment of diabetes, none is found to be ideal due to undesirable side effects and diminution after prolonged use. In the traditional system of Indian medicine, formulation with extracts of plant parts is used as the drug of choice as antidiabetic and lipid-lowering agents. Although phytotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny. Based on folkloric use, the present study was designed to evaluate the antidiabetic potential of *Gmelina arborea* is a fast growing deciduous tree. Male albino rats of the Wister strain weighing around 90-160 g were used for this study. The rats were 10-12 weeks of age can be used in this study. 100 mg of leaf powder were dissolved in 1000 ml of water. This solvent was used to treat the alloxan induced rats for 21 days. The present study reveals the changes in the level of HbA_1c, Glucose, Urea, Creatinine, Protein, Albumin, Globulin, SGOT, SGPT and ALP. The results of the present study indicate that the plant extract is nontoxic and possess antidiabetic activity.

Keywords: Gemlina arborea, Alloxan, $sHbA_1C$, Gucose, Urea, Creatine, Protein, Albumin, Globulin, SGOT, SGPT and ALP

1. Introduction
Diabetes mellitus is a clinical syndrome, a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced [1]. In 1675 the word diabetes comes from Latin diabetes, intended meaning "excessive discharge of for the disease. The word mellitus comes from the classical Latin word (i.e. sweetened with honey; honey-sweet) [2] All forms of diabetes have been treatable since insulin became available in 1921. Many herbal medicines have been recommended for the treatment of diabetes [3]. Herbal drugs are prescribed widely because of their effective, less side effect and relatively low cost. The International Diabetes Federation (IDF) estimated the global burden of diabetes was 366 million in 2011 and it would rise to 552 million by 2030 [1].

Gmelina arborea is a fast growing deciduous tree, occurring naturally throughout greater part of India. This tree is commonly planted as a garden and an avenue tree; growing in villages along agricultural land and on village community lands and wastelands. The root and bark of *Gmelina arborea* are claimed to be stomachic, galactagogue laxative and anthelmintic; improve appetite, useful in hallucination, piles, abdominal pains, burning sensations, fevers, 'tridosha' and urinary discharge. Leaf paste is applied to relieve headache and juice is used as wash for ulcer. Flowers are sweet, cooling, bitter, acrid and astringent. They are useful in leprosy and blood diseases. Ayurveda, it has been observed that Gamhar fruit is acrid, sour, bitter, sweet, cooling, diuretic tonic, aphrodisiac, alternative astringent to the bowels, promote growth of hairs, useful in 'vata', thirst, anaemia, leprosy, ulcers and vaginal discharge [4, 5].

2. Materials Methods
2.1 Plant Materials
The plant was collect from thammampatti, salem (DT), Tamil Nadu. The plant was identify, authenticator and the voucher specimen has been in our laboratory for the future reference. The leaves were shade dried, powdered and passed through a 40-mesh sieve, and kept in a well closed container for further extraction.

2.2 Plant Extraction
100 mg of leaf powder and 1000 ml of distilled water added to (1:10) prepare aqueous extract heat in 70 ^{0}C in ½ hrs then extract filtered after stored in brown bottle.

2.3 Experimental Animal
Male albino rats of the wiser strain weighing around 90-160 g were used for this study. The rats were 10-12 weeks of age can be used in this study. They were acclimatized to the animal house condition at least for one week before carrying out any experimental work. The rats were fed ad libitum with normal pellet and water. The ethical norms approved by ministry of social justice committee guideline for the investigation.

2.4 Experimental Induction of Diabetes
The rats were fasted for 18 hours and made hyperglycemic by a single intraperitoneal injection of alloxan dissolved in 0.09% NACL, at a 120 mg/kg body wt. The blood glucose levels of these rats were estimated 72 hours after alloxan administration, and alloxan diabetic rats having glycosuria (indicated by benedicts qualitative test) and moderate hyperglycemia (above 250 mg/dl) were selected for the experiment.

2.5 Experimental Design
The method described by Pari and Sathesh (2004) was adopted. In the experiment a total of 30 rats (18 diabetic surviving rats, and 12 normal rats) were used. The rats were divided into 5 groups (6 rats/group) after the induction of alloxan monohydrate.

Group 1: Normal untreated rats

Group 2: Normal rats were given with plant treatment

Group 3: Diabetic control

Group 4: Diabetic rats were given GA200/KG body weight in aqueous solution daily for 20 days

Group 5: Diabetic rats were given glipiside 200 ug/kg body weight (pariand Umamaheswari, 2000) in aqueous solution daily for 20 days.

2.6 Preliminary Phytochemical Screening
The aqueous extract of gmilina arborea leaves were subject to preliminary phytochemical test result for protein, carbohydrate, saponin, alkaloids, terpenoids were present and confirmed.

2.7 Oral Glucose Tolerance Test
At the end of the experimental period, fasting blood samples were taken from all the groups of rats to perform oral glucose tolerance test. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after an oral administration of glucose solution at a dosage of 2 g kg-1 body weight. All the blood samples were collected with EDTA for the estimation of glucose by O-toluidine method.

2.8 Biochemical parameters
Plasma insulin was assayed using the Ultra-sensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Hemoglobin and glycosylated hemoglobin (HbA1c) levels were estimated. Blood urea, serum creatinine and uric acid were also assessed. Urine sugar was detected using urine strips. Aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) were assayed.

2.9 Biochemical and Hematological Parameters
2.9.1 Statistical analysis
All the grouped data were statistically evaluated with SPSS 16.0 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference test. A value of $p<0.05$ was considered to indicate statistical significance. All results are expressed as mean ± SEM for six rats in each group.

3. Results
Glucose is a substrate and an indispensable energy supplier, which supports cellular function. Alloxan, a beta cytotoxin induce diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic cell, resulting in a decrease in endogenous insulin release, it leads to decrease utilization of glucose by the tissue. (Okamato *et al*., 1981).

Table 1: Preliminary Phytochemical analysis

S. No	Phytochemical Test	Aqueous Extract
1	Alkaloids	+
2	Carbohydrate	+
3	Glycoside	+
4	Anthraquinone	-
5	Protein and amino acid	+
6	Tannins	+
7	Phenolic compound	+
8	Steroids and sterols	+
9	Triterpenoids	+
10	Saponins and flavonoids	+

Table 2: Estimation of HbA1C, Glucose, Urine Sugar, Protein level of an experimental rats.

Group	HBA_1C	Glucose	Urine Sugar	Protein
Normal	16.01±28.21	140.83±3.18	Nil	7.03±0.60
Diabetic- control	20±26.15	303.50±37.51	+++	4.21±0.91
Plant treatment	16.48±28.01	132.50±4.72	+	7.20±0.40
Tablet treatment	16.55±27.97	117.66±22.33	+	7.15±0.53

Table-2 depicts the level of HbA1C, Glucose, urine Sugar in the experimental rats. The protein level was decreased in diabetic control compared to the normal rats. The levels of glucose and glycosylated haemoglobin were raised in diabetic control. After the administration of plant drug the level were significantly reduced and comes to near normal.

3.1 Phytochemical Analysis

Table-1 depicts the preliminary phytochemical investigation of the aqueous extract of *Gmelina arborea*. The presence of protein, carbohydrate, saponin, alkaloids, terpenoids were conformed.

Table 3: Estimation of Creatinine, Urea level of an experimental rats.

Group	Creatinine	Urea	Albumn	Globulin
Normal	1.18±0.21	37.00±3.34	4.26±0.51	2.65±0.60
Diabetic- control	2.96±0.34	47.16±1.72	2.31±0.29	0.95±0.18
Plant treatment	1.10±0.14	33.00±4.97	4.00±0.64	2.71±0.44
Tablet treatment	1.05±0.18	30.16±10.98	4.45±0.57	2.80±0.74

Table-3 depicts the level of creatinine, urea Albumin, Globulin in the experimental rats. All the levels were raised in diabetic control. After the administration of plant and tablet drug the level were significantly reduced. The level of albumin, globulin in the experimental rats was decreased in diabetic control. All the levels were increased in diabetic rats after the administration of plant drug.

Table 4: Estimation of SGOT, SGPT, ALP level of an experimental rats.

GROUP	SGOT	SGPT	ALP
Normal	29.83±8.30	36.16±8.20	18.85±19.76
Diabetic- control	59.66±2.58	55.16±2.63	20±26.15
Plant treatment	33.83±3.43	36.83±3.65	16.48±28.01
Tablet treatment	33.50±9.73	38.50±6.31	16.55±27.97

Table-5depicts level of SGOT, SGPT, ALP in the experimental rats. All the levels were increased in diabetic control. After the administration of plant drug the level were significantly reduced.

4. Discussion

In general, there is very little scientific knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain active ingredients such as flavonoids, alkaloids, glycosides, terpenoids, etc., which possess antidiabetic effects. Phytochemical analysis of the leaf extract revealed the presence of flavonoids, alkaloids, glycosides, polyphenols, tannins, saponins, phytosterols and triterpenes in the leaf extract [6, 7].

Phytocompound based strategies play a pivotal role in the prevention and treatment of diabetes. Polyphenolic compounds such as flavonoids contribute to increased plasma antioxidant capacity, decreased oxidative stress markers and reduced total and LDL cholesterol. Growing evidence indicates that various dietary polyphenols may influence carbohydrate metabolism at level in blood.

many levels. Phytochemicals include compounds with various biological properties which allow plants to cope up with environmental challenges including exposure to radiation and toxins. They are bioactive compounds (secondary metabolites) found in plants that works with nutrients and dietary fibers to protect against diseases. The presence of the biologically active ingredients in the fruit extract may account for the observed pharmacological actions [7].

The currently available drugs for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose

Alloxan is one of the usual substances used for the induction

of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas (Jelodar *et al*., 2003). Alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of langerhans, there by inducing hyperglycemia. The results in present study indicate that *gmelina arborea* leaf extract was found to reduce the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury.

In the present investigation aqueous extract of *gmelina arborea* leaf demonstrated the significant anti diabetic activity. The results from the study also indicate that *gmelina arborea* leaf extract can reduce the levels of serum urea, serum creatinine, serum cholesterol and increase the serum protein and confirms the possibility that the major function of the extract are on the production of vital tissues including pancreas, thereby reducing the causation of diabetes in the experimental animals.

Glycated hemoglobin or βN-1-deoxyfructosyl-hemoglobin (HbA1c) is the product of a non enzymatic reaction of glycation, namely a condensation between the aldehydic group of glucose and the amino group of the terminal valine in the β-chain of haemoglobin A1. The amount of HbA1c is strictly related to blood glucose concentration. Considering the average life span of red cells, the HbA1c value should mimic the mean glycemic value of the previous 2–3 months [8].

The American Diabetes Association (ADA) recommends HbA1c determination in patients with diabetes mellitus on therapy in order to monitor the glycol metabolic status in the medium–long term and thus reduce the risk of vascular complications. During diabetes, the excess of glucose present in the blood reacts irreversibly with amino groups of lysine residues in Hb to form HbA1c. Diabetic rats showed higher levels of HbA1c indicating their poor glycemic control. Oral administration of fruit extract to diabetic rats decreased the levels of glycosylated haemoglobin by virtue of its hypoglycemic activity. This normalization of HbA1c indicates decreased glycation of proteins and confirms the anti-diabetic potential of leaf extract [9].

The amount of urea in the blood is increased with concomitant decrease in plasma protein levels in experimental diabetes as a result of increased breakdown of plasma and tissue proteins due to negative nitrogen balance. Further, the supraphysiological concentration of glucose in diabetic state causes severe derangement in protein metabolism that result in the development of negative nitrogen balance. This in turn elevates urea and creatinine levels which acts as biochemical diagnostic markers for assessing renal impairment and drug-induced toxicity [10].

Serum creatinine concentration is often used as a variable not only to assess impairment of kidney function but also as clinical end point to detect treatment related toxic effects of compounds on the kidney in experimental animals. The observed alteration in the levels of blood urea and serum creatinine in group of diabetic rats reverted to near normalcy by treatment with fruit extract, indicating renal protective nature of the extract during glucose toxicity. The level of purines is elevated due to accelerated muscle wasting. These accumulated purines are the main source for the production of uric acid by the activity of xanthine oxidase [11, 12].

In the present study the activities of SGOT, SGPT and ALP in serum were altered in DM. In diabetic animals, the changes in the levels of SGOT, SGPT and ALP are directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis.

SGOT and SGPT levels also act as an indicator of liver function hence restoration of normal level of these enzymes indicates that the normal functioning of liver. Increased activities of serum ALP have been observed in alloxan induced diabetic rats [12, 13].

Overall results showing the antidiabetic activity of *gmelina arborea* leaves, may be due to presence of chemical constituents like flavonoids, terpenoids etc. [14].

5. Conclusion

From this study, we can state that the aqueos extract of *gmelina arborea* has beneficial effects on blood glucose level as well as improving hyperlipidemia and other metabolic aberrations. It has the potential to impart therapeutic effects in diabetes induced diabetic rats.

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