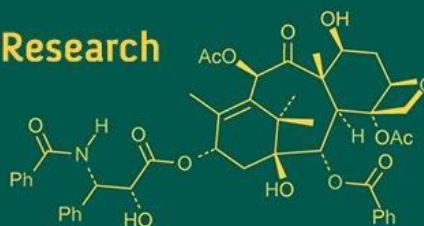


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(8): 410-415
www.biochemjournal.com
Received: 12-06-2025
Accepted: 15-07-2025

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Hypoglycemic and hypolipidemic effect of *Syzygium cumini* seed extract in alloxan induced diabetes mellitus in rats

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i8Sg.5187>

Abstract

The antidiabetic effects of aqueous seed extract of *Syzygium cumini* was examined in diabetes mellitus induced albino wistar rats using alloxan monohydrate, which was also compared with hypoglycemic and hypolipidemic effects of metformin. This drug is generally used in diabetes mellitus treatment in humans as well as canines. This selected drug was studied systematically, analyzed and compared with plant extract. For induction of diabetes, male albino wistar rats of average weight about 120 gm were intraperitoneally administered with alloxan monohydrate, a crystal pink powder, dissolved in cold NS, at the dose rate of 120 mg/kg BW. Alloxan was used in all rats except negative control group. Diabetes induction was confirmed by evaluating the blood glucose levels. The blood glucose level of selected rats for this study was about 160 mg/dL. Rats of Group I served as normal control and rats of Group II served as hyperglycemic control. Group III was given Metformin in 1 mL of aqueous solution at a dose rate of 400 mg/kg BW p.o., whereas Group IV was orally given *Syzygium cumini* seed extract at the dose rate of 0.25 g/kg BW diluted in 1ml aqueous solution. Group V was given orally 1ml of aqueous diluted *Syzygium cumini* seed extract at the dose rate of 0.50 g/kg BW and Group VI was given orally 1ml of aqueous diluted *Syzygium cumini* seed extract the dose rate of 0.75 g/kg BW. Alloxan induced diabetic rats of Group II showed increase in the levels of total cholesterol, serum triglycerides, serum creatinine, blood urea nitrogen and blood glucose till the end of this study. Group III, treated with standard drug metformin at a dose 400 mg/kg BW significantly reduced the elevated blood glucose level, total cholesterol, serum triglycerides, blood urea nitrogen (BUN); and serum creatinine level was restored near to the normal range. In group IV after administration of aqueous seed extract of *Syzygium cumini* at a dose 0.25 g/kg BW significantly reduced the elevated blood glucose level, total cholesterol, serum triglycerides, blood urea nitrogen (BUN), and serum creatinine level. In group V administration of aqueous seed extract of *Syzygium cumini* at a dose 0.50 g/kg BW significantly reduced the elevated blood glucose level, total cholesterol, serum triglycerides, blood urea nitrogen (BUN) and serum creatinine level. In group VI after administration of aqueous seed extract of *Syzygium cumini* at a dose 0.75 g/kg BW significantly reduced the elevated blood glucose level, total cholesterol, serum triglycerides, blood urea nitrogen (BUN); and serum creatinine level was restored near to the normal range. In this study, results showed that aqueous seed extract of *Syzygium cumini* showed both hypoglycemic and hypolipidemic activities, which can further be studied for the advancement of the drug discovery for the diabetes mellitus treatment.

Keywords: *Syzygium cumini*, diabetes, hypoglycemic, hypolipidemic, alloxan, metformin

Introduction

Diabetes mellitus is a metabolic disorder that can be fatal. There are multiple factors involved in disease aetiology; like unhealthy lifestyle habits, lesser physical work, stress, genetic link or beta cell damage due to some environmental toxicants like arsenic and dioxins etc. Chronic hyperglycaemia is characterized by disruption of normal carbohydrate, fat, and protein metabolism. Improper secretion of insulin, alteration in insulin action etc. may be the aetiological causes. International Diabetes Federation (IDF) noted incidences of diabetes globally as 239 million per year (Zimmet, 1999) ^[1]. Side effects are associated with sulphonylurea and biguanide therapy for diabetes. Chronic sulphonylurea therapy may inactivate pancreatic β -cells and high concentrations of sulphonylurea may inhibit both *in vitro* and *in vivo* insulin biosynthesis. Such drugs must be avoided during pregnancy. In the homeostasis of glucose and lipids, liver plays a fundamental role.

Enzymes involved in glucose metabolism are significantly altered in the experimental diabetes research and these altered enzyme levels are the cause of hyperglycaemia; contributing to the pathogenesis of diabetic complications that often adversely affect other body functions.

Typically, diabetes is classified into two categories; type I or insulin-dependent diabetes mellitus (IDDM) and type II or non-insulin-dependent diabetes mellitus (NIDDM). More accurate way to differentiate these two complications would be to classify insulin dependent diabetes as ketoacidosis effect and the non-insulin dependent diabetes as ketoacidosis resistant effect in the body. Based on the immune system, etiological grounds for type-I DM is immune-mediated condition, where as type-II DM is non immune mediated condition.

Most diabetic canines suffer from diabetes with insulin deficiency, with the underlying cause of an inflammatory process in the exocrine or endocrine tissue. Autoimmunity is suspected in some instances of self-apoptosis of the beta cells, being one of the main causes of pancreatic β -cell loss or death of the β -cell. Pancreatitis due to some infections, which may be diagnosed concurrently with diabetes in some cases, results in variation or loss of Islet cell of Lagerhans architecture. Canine islets cell of Langerhans have an average of 78% β -Cell producing unit of insulin production, 11% α -cells producing unit for glucagon production (Glucagon is a peptide hormone), $\leq 11\%$ δ -cells or D cells to secrete the hormone somatostatin, while in humans the islets have β -cell 50%, α -cell 40%, δ -cell 10%. The research findings in canines is in contrast with pathological findings, in which insulinitis is a distinguishing feature. Insulinitis is known to affect, through the presence of a lymphocytic infiltrate, the periphery (peri-insulitis) or the interior of the islet cells of the langerhan (intra-insulitis) in pancreas. Treatment with oral hypoglycemic agent such as Sulphonylurea are often associated with side effects such as desensitization of β -cell, GIT disturbances, skin reaction and rise in hepatic enzyme levels.

Various medicinal plants have been tested for antidiabetic effects in laboratory animals. Vat vriksha has many medicinal values that can be obtained from stem bark and fruits, also having antidiabetic property commonly known as Bargad, belonging to the family Moraceae shows hypolipidemic activity, antioxidant activity etc. The compound isolated from *Ficus benghalensis* Linn was 3'-5-dimethyl-ether of leucocyanidin-3, 0-p-O-D galactosulcellobioside. Some other plants available in Odisha showing antidiabetic property are *syzigium cumini* (jamun) and *Melia azadiracta*. There is an ample scope for development of new medicines from plant sources to combat the diabetic condition.

As diabetes is a chronic disease medicines used for this condition must not be hazardous. According to the World Health Organisation, in developing countries such as India, 70-80% of the population still depends predominantly on non-conventional medicinal sources in their primary health care; while some follow Ayurveda for treatment which has already suggested various plant sources as its medicine to tackle the chronic cases without side effects. For geriatric patients the herbal drugs are one of the most popular choice. Even in modern allopathy way of treatment, pharmacological screening of medicinal plants often provides a significant basis for the development of new lead molecules, a chemical entity that creates some active

pharmaceutical ingredient.

Biochemical change associated with diabetes mellitus

An inability to regulate blood glucose is a hallmark trait of diabetes mellitus. The triphasic blood glucose curve in alloxan-diabetic rabbits was documented by Bailey and Bailey (1943) ^[10]. The phases were marked with early hyperglycemia lasting 2-3 hours; 6-12 hours long hyperglycemia and ultimately chronic hyperglycemia. In addition to the observations of Bailey and Bailey (1949) ^[11], Shiplay and Beyer (1947) ^[6] noted a small initial decrease in blood glucose levels at 15 to 30 minutes and designated the additional curve as tetraphasic. A typical characteristic of hyperglycemia is both clinical (Taylor and Agius, 1987) ^[7] and experimental diabetes mellitus (Rerup, 1970) ^[8]. Tomlinson *et al.* (1985) ^[9] reported that diabetic rat's blood glucose level was 3 to 4 times higher than those of normal rats

Materials and Methods

White male albino rats of wistar strain weighing about 120-160 g procured and were housed in well ventilated, solid bottom polypropylene cage at normal room temperature. All animals received laboratory pellet feed and water was provided ad libitum. Animals were acclimatized to the laboratory conditions in laboratory animal facility of the Veterinary Pharmacology Department, College of Veterinary Science and Animal Husbandry OUAT, Bhubaneswar, Odisha for 1 week before being used for the experiment. The experimental protocols were accepted by the committee for institutional animal ethics (IAEC).

Chemicals and Drugs

Alloxan monohydrochloride: (SRL laboratories Pvt. Ltd.). Normal saline: (Claris life science Ltd., Ahmedabad) 20% Dextrose normal saline: (Parental drug limited, Indore). Glucose Solution: 5 g glucose (SD fine chemicals) dissolved in 100 ml of distilled water Rat bleeding capillaries: (Pearls agencies, Hyderabad) Agappe diagnostics LTD. Creatinine serum analysis kit: Ernakulum, Kerala, Biosystem biochemicals cholesterol kit: Irungattukottai, TN. Glibenclamide (Daonil®): Snofi India limited, Ankleshwar, Controle D glucometer strips and digital meter. Ketamin hydrochloride (Aniket®): Neon pharmaceuticals. Tryglycerides Serum Kit: Coral clinical systems, Blood urea nitrogen serum analysis kit: Coral clinical systems, Swent feeding needle set: Jamnagar, GJ, *Syzygium cumini* seed from D.K agrotech Delhi.

Experimental Design

Blood was collected from all the animals and blood glucose concentration was estimated to rule out spontaneous diabetes. Animals which showed a mean blood sugar level of about 60 to 80 mg/dL were selected for the study. From these animals 30 wistar albino rats with a prior fasting of 18 h, were injected with alloxan monohydrochloride (120 mg/kg *i.p* in NS), while 6 animals were injected with normal saline which were used as negative control of group I. To avoid fatal hypoglycemia due to initial massive pancreatic insulin release, animals were given 20% glucose (D20) solution orally (ad lib, in feeder bottles) after 6h of alloxan injection. The rats were then kept for the next 24 h on 5% glucose (D5) solution in drinker bottles in their cages to prevent hypoglycemia. After 72 h, alloxan treated rats with

a mean blood glucose level above 170 mg/dL or above were selected for the study and were divided into five groups of 6 animals each. Group I served as normal control group to which there was no induction of diabetes mellitus, Group II as hyperglycemic positive control to which no other medication was given except NS i.p after induction of diabetes by alloxan monohydrate. Rats of group III were given Metformin Glycomet® at dose rate of 400 mg/kg BW p.o by intra gastric needle in 1mL of aqueous solution. Rats of group IV, V and VI were given aqueous diluted *Syzygium cumini* orally as 1mL aqueous solution, as a single dose (g/kg, BW) for 28 days @ 0.25, 0.50, and 0.75, or standardized dose administration by the use of micropipette at 30 µl, 60 µl and 90 µl respectively.

Group I: Normal control: Rats of this group were fed on basal diet without any treatment

Group II: Diabetic control: Rats of this group were induced diabetic models and were served in diabetic control

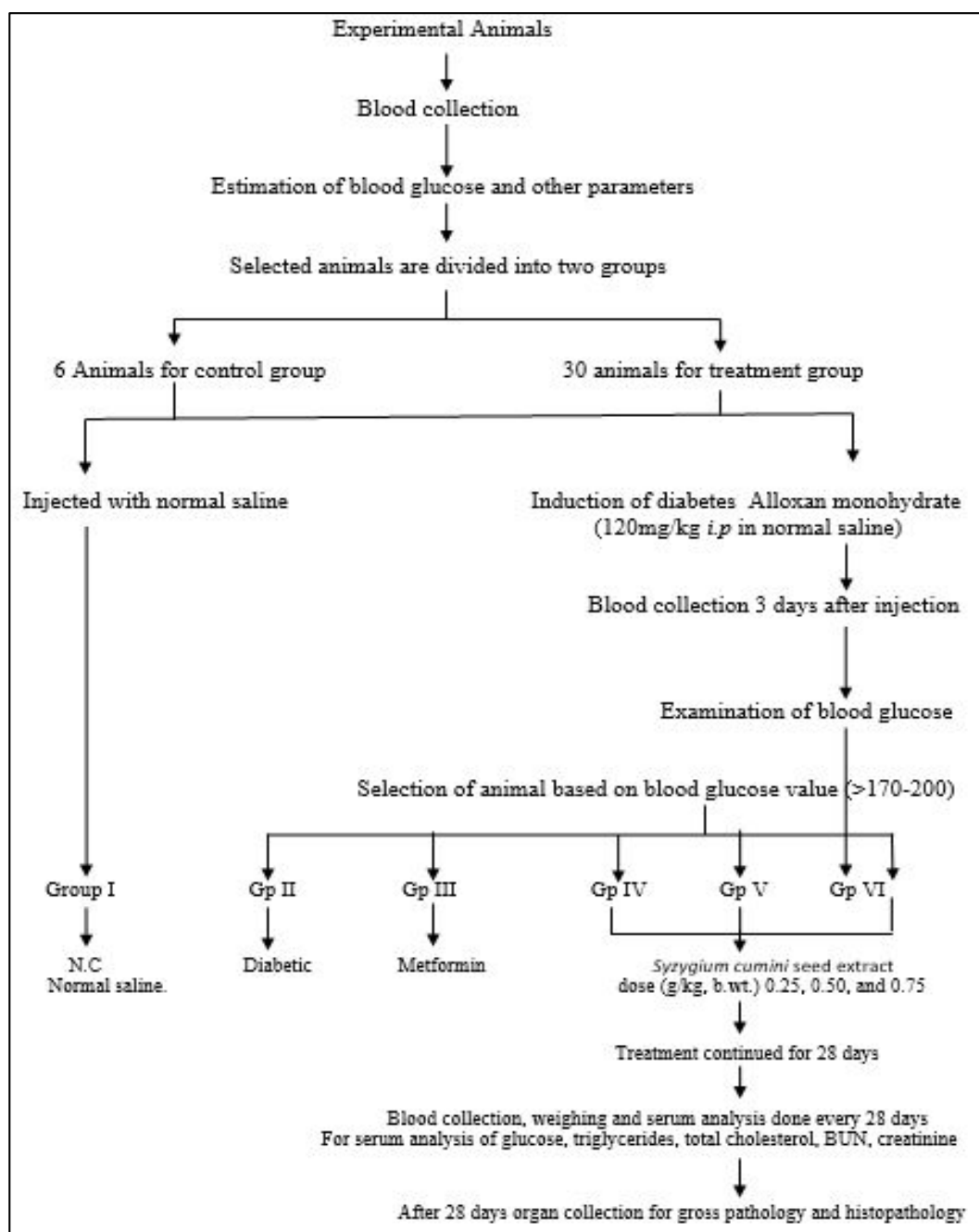
throughout the experiment without the therapy

Group III: Diabetic models of rats with standard drug metformin. Rats of this group orally gavaged with 400 mg/kg BW of Metformin (Glycomet®)

Group IV: Were given aqueous diluted *Syzygium cumini* orally as 1mL aqueous solution as a single dose (g/kg, BW) for 28 days @ 0.25 or standardized dose administration by the use of micropipette at 30 µl.

Group V: Were given aqueous diluted *Syzygium cumini* orally as 1mL aqueous solution as a single dose (g/kg, BW) for 28 days @ 0.50, or standardized dose administration by the use of micropipette at 60 µl.

Group VI: Were given aqueous diluted *Syzygium cumini* orally as 1mL aqueous solution as a single dose (g/kg, BW) for 28 days @ 0.75, or standardized dose administration by the use of micropipette at 90 µl.



Serum biochemical estimations

Blood was collected under light ether anesthesia by using heparinized capillary tube. Approximately 1.0 mL of blood was collected from tail vein into heparinized eppendorf tubes. Immediately after blood collection the samples were centrifuged in micro centrifuge machine (Hetitech-Microcentrifuge) at 3000 rpm for 15 minutes at 15 °C. The clear supernatant plasma was separated for estimation of glucose, total cholesterol, triglycerides, serum creatinine, blood urea nitrogen using diagnostic kits.

Statistical analysis

The data was subjected to two way ANOVA as per the methods described by Snedecor and Cochran (1994) [12]. $p < 0.01$ was considered as significant.

Results

Syzygium cumini seed extract in alloxan induced diabetic wistar albino rats compared to conventional drugs available in the market such as metformine are described below.

Plasma glucose concentration in groups IV, V and VI treated with *Syzygium cumini* seed extract at 0.25, 0.50 and 0.75 g/kg BW respectively showed a significant ($p < 0.01$) reduction when compared to diabetic control group II starting from the day 7 and the trend continued in a similar manner on day 14, 21 and at 28 also. Since no reports have been published on the hypoglycaemic properties of *Syzygium cumini*, the following concept could not be compared with previous studies, but the reduction in rat plasma glucose concentration in the present study was consistent with the previous study on seeds of *eugenia jambolana*; a plant pertaining to the same Myrtaceae family (T. Gohil *et al.*, 2010) [3].

Table 1: Mean plasma glucose concentrations in mg/dl

Group	B. value	0 day	7 day	14day	21day	28day
G1	58	62	68	69	70	72
G2	60	274	270	269	250	240
G3	59	272	210	170	161	110
G4	60	264	222	188	152	134
G5	58	255	202	182	144	110
G6	61	248	206	161	104	98

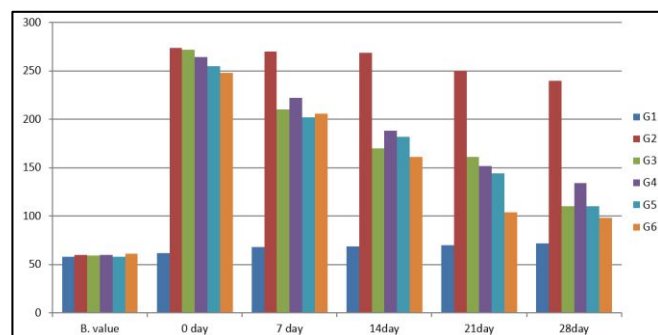


Fig 1: Mean plasma glucose concentration in mg/dl comparison group wise

5.2 Effect on Lipid profile

Decrease in the insulin concentrations of diabetic animals has a profound effect on lipid metabolism. Insulin enhances lipoprotein lipase transcription in the capillary endothelium. This enzyme hydrolyzes triglycerides and chylomicrons present in the VLDL, resulting in the release of LDL particles. The liver transmits the LDL particles to the

cholesterol rich HDL. Thus, hypertriglyceridemia and hyper-cholesterolemia often occur in untreated diabetic patients due to the reversal of all of the above consequences, so the lipid profile was evaluated to test the drug's efficacy in experimental diabetes.

The lipid profile revealed significant ($p < 0.01$) increases in total cholesterol, where as LDL showed a noticeable reduction.

5.2.1 Effect on total cholesterol

A significant ($p < 0.01$) increase in plasma cholesterol concentration was observed in alloxan induced diabetic rats. This rise in plasma cholesterol levels was in accordance with the suggestion of Bopanna *et al.*, (1997) [12]. Total cholesterol concentration of the group treated with aqueous diluted *Syzygium cumini* seed extract showed a significant ($p < 0.01$) decrease when compared with diabetic control group II on day 7 and this reduced values continued until subsequent observations on day 14, day 21 and day 28 in a dose-dependent manner.

Table 2: Mean plasma total cholesterol concentrations in mg/dl in rats.

Group	B. value	0 day	7 day	14 day	21day	28 day
G1	73	73	75	72	76	74
G2	74	120	136	132	138	140
G3	74	104	102	95	92	90
G4	73	117	104	92	95	92
G5	74	116	110	96	94	89
G6	76	114	108	92	90	88

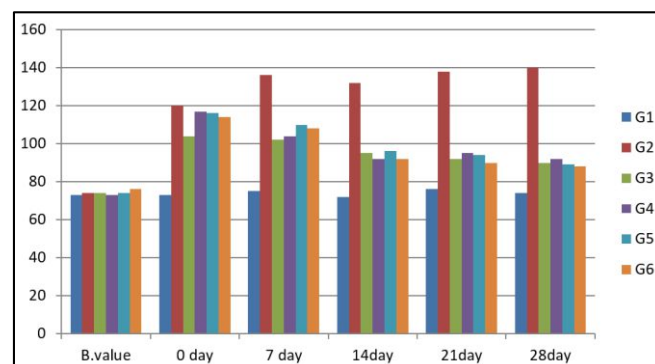


Fig 2: Mean plasma total cholesterol concentration in mg/dl in rats comparison group wise

Triglycerides

In NIDDM the triacylglycerolemia may be due to increased production of triacylglycerol by the liver. The *Syzygium cumini* treated groups of rats showed significant decrease in plasma concentration of triglycerides on day 0, 7, 14 and 28 days. Fatty acid esterification in the liver results in the formation of triacylglycerol, which can be obtained from dietary carbohydrates, amino acids and reserves of stored adipose tissue. Triacylglycerol is stored inside the cell or can be combined with apoproteins and secreted in the form of VLDL. With increased fatty acid concentration due to diabetic conditions, the fatty acid esterification process can be increased; this results in the presence of nonsaturable fat in the circulation, whereas the presence of apoproteins or other lipoprotein particles may limit the VLDL concentration. Whenever VLDL saturation is achieved then triacylglycerol accumulation process starts in the liver. The elevated plasma triacylglycerol, in diabetes is generally

associated with VLDL but it also increases other lipoproteins level such as LDL and HDL levels (Andallu B *et al.*, 2009) ^[4].

Table 3: Mean plasma concentration of serum triglycerides (mg/dl) in rats.

Group	B.value	0 day	7 day	14day	21day	28day
G1	42	46	45	46	43	44
G2	44	97	104	114	126	120
G3	46	58	54	48	44	43
G4	46	67	65	64	64	60
G5	48	68	66	62	61	60
G6	48	70	68	64	62	58

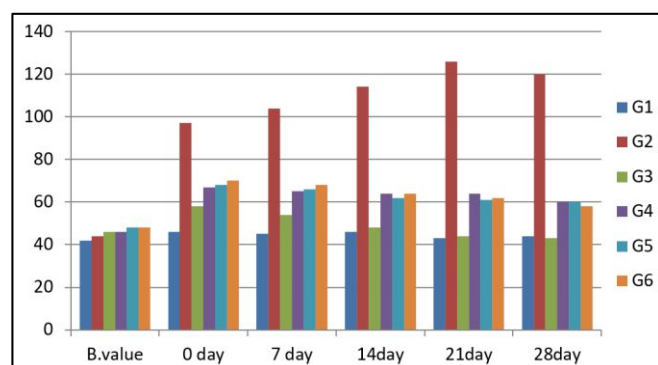


Fig 3: Mean plasma concentration of serum Triglycerides in rats comparison group wise

Discussion

The elevated blood sugar levels in diabetes mellitus can be due to either cell insulin resistance or decreased insulin secretion from β -cells that may manifest in the decreased serum insulin levels. Hyperglycemia in the study was induced by alloxan monohydrate which cause destruction of β -cells of the islets of Langerhans so there is massive decline in insulin release and results in a reduction of serum insulin levels. Both glucose and lipid metabolism are adversely affected in diabetes. Serum insulin in the postprandial (after meal) state increases the activity of lipoprotein lipase in adipose tissue and acts as fuel storage in normal metabolism in the form of triglycerides. Insulin deficiency decreases lipoprotein lipase activity, thereby leading to the deranged metabolism of lipoproteins. In the current study, lipoprotein levels in diabetic rats induced by alloxan monohydrate showed a significant alteration in lipoprotein metabolism. In diabetic animals, serum total cholesterol level increased significantly. Maintaining blood glucose levels help to prevent renal problems such as chronic liver failure and diabetic nephropathy. Progressive diabetes mellitus can be prevented by systemic analysis of serum creatinine and blood urea nitrogen. These tests are very useful in diabetes patients for the assessment of kidney function. Some of the indigenous medicinal plants found to be better options in managing diabetes; and amongst them, *Syzygium cumini* was used for this study. This selected plant was evaluated in the present experimental study. Diabetes was induced by injecting freshly prepared alloxan solution diluted in 0.9% NS, which was light protected, at dose levels 120mg/kg BW intraperitoneally to the overnight fasted animals in semi dark room. The single dose was injected with a total volume of 1ml. Certain biomarkers were evaluated for the any change. *Syzygium cumini* seed extract at three different doses of 0.25 g/kg BW, 0.50 g/kg

BW and 0.25 g/kg BW, respectively. The obtained results are summarized as follows.

Plasma glucose concentration except that of normal control (Group 1) revealed a significant ($p < 0.01$) increase after 72 hr of administration of alloxan (Day 0) as compared to the basal values. There is significant ($p < 0.01$) decline in concentration of plasma glucose levels in rats treated with *Syzygium cumini* seed extract. One standard drug used in the study, metformin also showed reduced concentration of plasma glucose levels. The blood glucose levels started declining from Day 10, and were consistent throughout the experiment. It was noticed that till the end of trial period of 28 days aqueous seed extract of *Syzygium cumini* at 0.75 gm/kg BW was nearly as effective as metformin, the other standard drug used to lower glucose levels, but the levels never corresponded to normal levels.

The lipid profile assessment in induced diabetic rats showed a significant increase in total cholesterol and triglycerides ($p < 0.01$) on Day 0 compared to basal values. The total levels of cholesterol and triglycerides have been decreased significantly for, *Syzygium cumini* seed extract and the standard drug metformin ($p < 0.01$). From day 10 it was observed that the reduction was consistent throughout the experiment, but the level of total cholesterol and triglycerides did not match with that of the normal control group rats.

The biomarkers for renal profile BUN and creatinine were increased significantly ($p < 0.01$), following induction of diabetes in all the group of rats except the normal control group I. Following treatment with the plant extracts of, *Syzygium cumini* and standard drug metformine, it was observed that the level of BUN and creatinine reduced significantly ($p < 0.01$) in the respective treatment groups, though they did not match with the level of control group.

A significant ($p < 0.01$) and consistent reduction in body weight of the diabetic control group rats was observed by measuring weight weekly. A significant ($p < 0.01$) and consistent increase in body weight was found in the group treated with plant extract of *Syzygium cumini* seed extract and standard drugs, and the increase in weight was highly significant ($p < 0.01$) for the normal control group.

Clinical examination of rats after DM induction revealed symptoms like dullness, anorexia, polyuria, dehydration, polydipsia, emaciation and weakness which may be due to due to impairment in carbohydrate, fat and protein metabolism associated with complete, relatively low insulin secretion or hampered insulin activity. In the diabetic control group II rats, there was depression and loss of general body condition. Groups treated with *Syzygium cumini* seed extract and standard drugs metformin showed greater improvement and were apparently normal by day 20. The rats of the normal control groups were maintaining proper health throughout the experiment.

The results of the current study have showed that the administration of aqueous *Syzygium cumini* seed extract had a significant ameliorative effect with an efficacy comparable to metformin against the adverse effects of alloxan induced diabetes. The probable anti-diabetic action of *Syzygium cumini* seed may be because of restoration of pancreatic β -cells, reduced production of hepatic triglycerides and reduced fat breakdown. Lipolysis can be due to elevation of serum insulin level in the plant extract treated rat groups but still detailed studies are required to get the authentic conclusions on the mode of pharmacological actions of

antihyperglycemic effect of both the plants *Ficus benghalensis* Linn bark laticiferous sap and *Syzygium cumini* seed.

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