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Toxicity Study and Anti-Diabetic Activity of Indeginious Traditional Medicinal Plant *Syzygium Cumini*

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Abstract

Syzygium cumini is used as traditional medicines to treat diabetes mellitus. The current study is done on treatment of diabetes mellitus with various herbal plants of Maharashtra region. Initially acute toxicity studies were performed, and diabetes is induced in rats with alloxane and streptozocin in laboratory. Alloxan induced diabetes is like type I diabetes due to deficiency of insulin in the body because of no release of insulin and type II diabetes is studied on the basis of hyperglycemia or obesity of the patients. These are treated with different plant extracts and comparison is done with standard antidiabetic drug.

The most important plant syzygium cumini is selected from Maharshtra vidharbha region and this is also found all over the country and most widely utilized for controlling sugar and lipid profile. Here these plants and their toxicity were studies for antidiabetic purpose. Jamun plant fruits and seeds are useful constituents for extraction and isolation of active constituents.

Keywords: diabetes, syzygium cumini, extraction and evaluation, toxicity, anti-diabetes evaluation.

1. Introduction

Plants are very important in our daily life without them we cannot live because they provide lot of thing for running of life like fuel, medicine, vegetable, wood, fruits, seeds, rain, food etc. food grains are obtained from wheat, millet, barley, gram, rice, groundnut, maize, guar, sorghum and many types of other plants. Woods are very important for furniture, shelter, preparation and cooking of food and vegetables, preparation of boat and paper. There are many plants that give us very valuable medicines like reserpine is obtained from root of Rauwlfia serpentine used in the treatment of hypertension and schizophrenia, quinine from bark of cinchona is used in the treatment of malaria, atropine is obtained from leaves of Atropa belladonna and used in dilation of pipuls from the time of beautiful lady Belladona, strychnine is obtained from root of nux vomica and used in the treatment of depression and central nervous system stimulant. There are lot of plants are used in the treatment of diabetes mellitus like fruits and seeds of jamun, karela and methi. The leaves of gemnema and jamun. These plants are easily available in Maharashtra and their water or alcoholic extracts are most powerful for treatment of hyperglycemia. Azadiractaindia is most precious plant and their leaves are useful for treatment of high sugar in urine.

Diabetes can be induced by different methods using streptozocine and alloxane. This can be generated with adrenaline injections in rats and other animals. The plants parts are collected,

and water or alcohol suitable solvent is used for extraction and administered to animals and three groups are made for measuring anti-diabetic activities. These are standard, test and control groups. Toxicity studies are also performed on the animals for safety purpose and drugs may be developed as ayurvedic formulation for better used in treatment and prevention of diabetes mellitus.

Sugar is a major complication at older age. Overeating and not controlling diet is major cause of diabetes. The pancreas release insulin but that insulin is not sufficient for digestion of more food so extra insulin is required. In old age cells are inactive and insulin is not secreted after requirement of our body. So, more sugar comes to the blood and reabsorbed by kidney. Pressure on the kidney is another complication and dangerous to the body. A regular load of sugar is the major cause of kidney failure. So regular checkup of body is beneficial and diet control is also required.

2. MATERIALS AND METHODOLOGY

Plant profile: *Syzygium Cumini*

Common names – Jamun
Family – Myrtaceae

Biological Source – It is fruit of *Syzygium cumini*, known as Jamun and black plum.
Medicinal uses- It is mainly used in diabetes as hypoglycemic agent. Fruit and seeds are utilized for this purpose. Fruits are purple in colour and generally flower in the rainy season. The plant is huge with 10-to-15-meterheight and available easily in India. There are lots of medicinal and pharmaceutical applications in hyperglycemia, hyperlipidemia, and other cardiovascular diseases. These fruits are extracted with the help of water and alcohol. These extracts are known as aqueous and alcoholic extract and utilized for treatment of sugar and obesity. The juice of syzygium cumini is very significant to sugar patients and it reduces sugar level directly from blood and beneficial to patients.

Adults without diabetes as recommended by the Organization for Economics and Development's recommendations (AOT no. 425), acute toxicity experiments were conducted on male albino rats. For the following 48 hours, the rats' behavioral, neurological, and autonomic profiles, as well as any signs of fatality, were monitored continually.
Alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg of body weight was administered intra peritoneally (IP) into the rats. Due to the potential for fatal hypoglycemia produced by the substantial mice had pancreatic insulin secretion administered a 15–20 ml of an intraperitoneal 20% glucose solution 6 hours after receiving alloxan. For an additional 24 hours, the rats were kept on bottles of 5% glucose solution to prevent hypoglycemia.
Chronic treatment model

A total of 60 rats (n = 6 groups of 5) were used in this study. The first group was the healthy controls, while the second was the untreated diabetics. Groups 3, 4, and 5 were administered 100 mg/kg, 200 mg/kg, and 500 mg/kg of ethanolic extracts of Cuminum cyminum and Trigonella foenum-graecum, and Chrysospermum mmi and Cicer arietinum L, respectively. Group 6 served as a control and received glibenclamide at 5 mg/kg daily for twenty-one days. In this study, participants' blood sugar levels and weights were measured on days 1, 7, 14, as well as 21.
Six groups of diabetic rats (n = 5) were created,

- Group I- Tween 80 in purified water at 5% (5 ml/kg b.w., p.o.) was given to normal control rats.
- Group II - Rats with diabetes were given an intraperitoneal (p.o.) solution of Tween 80 (5% in water).
- Group III - Oral administration of a herbal formulation (SGFS) at 100 mg/kg body weight was used to treat diabetes in rats.

- Group IV- herbal formulation (SGFS) 200mg/kg body weight, orally administered, was given to diabetic rats.
- Group V - herbal formulation (SGFS) 500mg/kg body weight, orally, was administered to diabetic rats.
- Group VI - Glibenclamide (5mg/kg body weight, orally) was administered to diabetic rats.

For a total of 21 days, extracts were given once daily. The Accu-check glucometer was used to determine blood glucose levels from blood samples taken via the lateral tail vein on days 1, 7, and 21 following medication delivery.

Oral feeding needles were used for all medication administration.

Acute study

Studies on the acute toxicity of a herbal preparation in rats. No graphical indication of abnormalities was identified up to 4 hours, as well as no deaths were recorded up in creatures until at the highest tested dosage level of 2000 mg/kg body weight after 48 hours. Such was then utilized as the MTD, as well as a test dose of 1/10th of the MTD was selected (100 mg/kg b.w.); moreover, a test dose of 500 mg/kg b.w. was selected for the experimental trials.

Subacute study

The medications were given to the animals at regular intervals over the course of 28 days. On days 1, 7, and 21 GLs were estimated. After 21 days, patients were given a week off from taking their medication to recover. On day 28, we made our best guess at the GLs. Average GL SEM was used to summarize the data.

Statistical analysis

The standard deviation and mean were used to summarize the data. Statistical examination was carried out in Graph Pad Prism 5 employing a one-way analysis of variance (ANOVA) as well as t-test of Dunnett. Statistical significance was determined by a probability value of less than *p0.05, p0.01, or p0.001 when compared to the control as well as reference groups, respectively. (Diabetes & Metabolism, 1989).

3. Results and Discussion

Toxicity Studies

Acute Oral Toxicity Study (72 hours)

50 % ethanolic extract of selected plant *Syzygium cumini* fruits (SGCF) &*Syzygium cumini* seeds (SGCS) were evaluated for its acute oral toxicity in mice. Animals were divided into five groups of two mice each weighing about 20-25 g. acute oral toxicity was conducted in three sets of experiments.

In first experiment, 50% ethanolic extracts of *Syzygium cumini* fruits (SGCF) & in second experiment *Syzygium cumini* seeds (SGCS) respectively were studied for their acute oral toxicity effects in mice. For this total thirty mice were taken & in each set of experiment, ten mice were divided into five groups of two mice each & groups were as follows: -

Group I ----- Normal Control. (2% gum acacia)
Group II ----- SGFS (500 mg / kg b.w)
Group III ---- SGFS (1000 mg / kg b.w)
Group IV ---- SGFS (1500 mg / kg b.w)
Group V ---- SGFS (2000 mg / kg b.w)

Mice were acclimatized for a period of 7 days before the start of treatment.

Ethanolic extract of SGFS in four dose levels was given orally in single doses to mice of Groups II, III, IV, V while Group I received only vehicle. (2% gum acacia). Extracts were administered in 2% gum acacia. animals were observed for mortality & general behaviour periodically, for 48 hr to 72 hr. behaviour of animals was observed daily for 1 hr in forenoon (10 to 11.am) animals were observed continuously for initial 4 hr. & intermittently for next six hr. & then again after 24, 48 & 72 hrs following administration of different doses of SGFS extract. Same procedure was followed for carrying acute oral toxicity study of 50% ethanolic extracts of Syzygium cumini fruits (SGCF) in which again ten mice were divided into five groups of two mice each.

Group I ----- Normal Control. (2% gum acacia)
Group II ----- SGCF (500 mg / kg b.w)
Group III ---- SGCF (1000 mg / kg b.w)
Group IV ---- SGCF (1500 mg / kg b.w)
Group V ---- SGCF (2000 mg / kg b.w)

Syzygium cumini seeds were also administered in same way in which again ten mice were divided into five groups of two mice each.

Group I ----- Normal Control. (2% gum acacia)
Group II ----- SGCS (500 mg / kg b.w)
Group III ---- SGCS (1000 mg / kg b.w)
Group IV ---- SGCS (1500 mg / kg b.w)
Group V ---- SGCS (2000 mg / kg b.w)

Following parameters were observed during acute oral toxicity study

- *Grooming* was considered in mice if animal cleared fur & skin of itself or another animal.
- *Hyperactivity* if there was any abnormal or excessive activity & animal was unable to relax
- *Sedation:*if animal was calm & composed without any stress
- *Having respiratory arrest*, if there was raising of head
- *Having convulsions* if there was tremor in tail or paddling of feet
- *Motor activity*, increased or decreased,
- *Mortality,* if any.

Subacute Toxicity study (14 days)

Ethanolic extract *Syzygium cumini* fruits (SGFS) were administered orally once daily to mice. Before initiation of experiment, mice were acclimatized for period of seven days. To study subacute

Toxicity, animals of either sex (20-25 g body weight) were divided into five groups of

Six mice each. Treatment was given as per following protocol.

Group I- Normal Control (2% aqueous gum acacia)

Group II SGFS (200 mg/kg b.w)

Group III SGFS (400 mg/kg b.w)

Group IV SGFS (250 mg/kg b.w)

Group V SGFS (500 mg/kg b.w)

Treatment was continued for 14 days. During this period, mice of control group received only 2% gum acacia. After 14 days, animals were fasted overnight & blood was collected by cardiac puncture. Blood samples were taken for haemoglobin & white blood corpuscles estimation. blood was allowed to clot for one hour & serum was separated by centrifuging & evaluated for different biochemical parameters results obtained were subjected to ANOVA followed by students t test, $p > 0.05$ was considered as non significant, $p< 0.05$ – significant, $p< 0.01$- highly significant & $p<0.001$ as very highly significant. After taking blood samples, animals were sacrificed. Liver kidney & spleen were excised from animals, preserved in 10% formalin & sent for histopathological studies. Following biochemical parameters were evaluated in subacute toxicity studies.

a) Serum Glucose Levels (Trinder *et al.*, 1969)

b) Kidney Function Tests

i. Serum Urea Levels (Varley, 1980)

Ii. Serum Creatinine Levels (Zender, 1972)

c) Liver Function Tests

i. Serum Bilirubin Levels (Jendrassik*et al*., 1938)

Ii. Serum Glutamate Oxaloacetate Transaminase (SGOT) (Reitman &Frankel, 1957)

Iii. Serum Glutamate Pyruvate Transaminase (SGPT) (Reitman & Frankel, 1957)

Iv. Serum Total Proteins (Lowry *et al*., 1951)

v. Serum Albumin (Hallbach*et al.*, 1991)

Vi. Serum Alkaline Phosphatase (Bowers *et al.,* 1972)

d) Blood Function Tests

i. Hemoglobin Value (Ghai, 2006)

ii. WBC Count (Ghai, 2006)

ANTIDIABETIC STUDY

Study of 50% ethanolic extract Syzygium cumini fruits (SGCF) & Syzygium cumini seeds (SGCS) against alloxan induced diabetes (10 days study)

In this study, ethanolic extract of SGFS (100 & 200 mg/kg b.w), SGCF (50,100 & 200 mg/kg b.w) & SGCS (100 & 200 mg/kg b.w) were evaluated for antidiabetic activity against alloxan induced diabetes mellitus in rats. Rats were divided into 10 groups consisting of 6 rats in each group. Initially 65 rats were taken to account for any mortality. Rats were acclimatized for period of 7 days before starting experiment. After overnight fasting, hyperglycaemia was induced by administering single dose of alloxan monohydrate supplied by s.d Fine-Chemical Ltd. Mumbai, India (120 mg/kg b.w) **(Vivek *et al.*, 2010)** prepared in sterile saline to all groups except group I which served as normal control. During this period, animals were given free access to water. After 5 days of alloxan administration, fasting blood glucose levels of rats were checked by glucostrips. Animals having blood glucose levels > 250 mg/dl were separated & selected for further studies & then re-grouping of these hyperglycemic rats was done as per following

protocol, for studying antidiabetic activity of different extracts.

Group I Normal Control (2% of gum acacia.)
Group II Diabetic Control (Alloxan monohydrate & 2% gum acacia)
Group IIIAlloxan monohydrate + Glibenclamide (10 mg/kg.).
Group IV Alloxan monohydrate + PO (100 mg/kg b.w)
Group V Alloxan monohydrate + PO (200mg/kg b.w).
Group VI Alloxan monohydrate + SGCF (50 mg/kg b.w)
Group VII Alloxan monohydrate + SGCF (100 mg/kg b.w)
Group VIII Alloxan monohydrate + SGCF (200 mg/kg b.w)
Group IX Alloxan monohydrate + SGCS (100 mg/kg b.w)
Group X Alloxan monohydrate + SGCS (200 mg/kg b.w)

Treatment was started from same day except normal control & diabetic control groups for period of 10 days orally. During this period, animals in all groups had free access to standard diet & water. Blood glucose levels were estimated on 1st, 4th, 7th & 10th day of treatment. Besides this, during this study the body weight of rats were recorded on 1st, 4th 7th & 10th day of treatment. On the 11th day, blood samples were collected from overnight fasted rats by cardiac puncture. Animals were anaesthetized by mild ether anaesthesia before cardiac puncture. Blood was collected & allowed to stand for one hour, serum was separated by centrifuging & evaluated for different biochemical parameters. Like

a) Serum Glucose Levels (Trinder *et al*., 1969)

b) Lipid Profile

i. Serum Total Cholesterol Levels (Allain *et al.,* 1974)
ii. Serum Triglycerides Levels (Bucolo, 1973)
iii. Serum HDL Cholesterol Levels (Izzo *et al*., 1981)
iv. Serum LDL Cholesterol Levels (Friedewald*et al.,* 1972)
v. Serum Total Protein Levels (Lowry *et al*., 1951)

c) Kidney Function Tests

i. Serum Urea Levels (Varley, 1980)
ii. Serum Creatinine Levels (Zender, 1972)
iii. Serum Total Protein Levels (Lowry *et al*., 1951)

d) Body Weight.

Study of 50% ethanolic extract of Syzygium cumini fruits (SGCF) & Syzygium cumini seeds (SGCS) against Streptozotocin (STZ) induced diabetes (15 days study)

In this study, ethanolic extract of SGFS (50 & 100 mg/kg b.w), *Syzygium cumini* fruits SGCF (50 & 100 mg/kg b.w) &*Syzygium cumini* seeds SGCS (50 & 100 mg/kg b.w) were evaluated for antidiabetic activity against streptozotocin induced diabetes mellitus in rats. Rats were divided into nine groups consisting of six rats each. Initially sixty rats were taken to account for any mortality. Rats were acclimatized for period of 7 days before starting experiment. After overnight fasting, hyperglycaemia was induced by administering single dose of streptozotocin. (50 mg/kg bow) (Prasad *et al,* 2009)to all rats excepting group I which served as normal control. Streptozotocin was freshly dissolved in 0.1 M citrate buffer(pH=4.5) & injected intraperitoneally within 15 min of dissolution in vehicle volume of 0.4 mL with 1 mL of tuberculin syringe fitted with 24 gauge needle. Diabetes was confirmed by determination of fasting glucose concentration on third day post administration of streptozotocin. During this period, animals were given free access to water. After 3rd day of STZ administration, fasting blood glucose levels of rats were checked by glucostrips. Animals having blood glucose levels > 250 mg/dl were separated & selected for further studies & then re-grouping of these hyperglycemic rats was done as per following protocol, for studying anti-diabetic activity of different extracts.

Rats were given following treatment in this study.

Group I Normal Control (2% gum acacia).
Group II Diabetic Control. Received STZ (50 mg/kg b.w single dose i.p)
Group III STZ + Glibenclamide (3 mg/kg,)
Group IV STZ + SGFS (50 mg/kg b.w)
Group V STZ + SGFS (100mg/kg b.w)
Group VI STZ+ SGCF (50 mg/kg b.w)
Group VII STZ+ SGCF (100 mg/kg b.w)

Group VIII STZ + SGCS (50 mg/kg b.w)
Group IX STZ + SGCS (100 mg/kg b.w)

Treatment was started from same day except normal control & diabetic control groups for period of 15 days orally. During this period, animals in all groups had free access to standard diet & water. Blood glucose levels were estimated on 1st, 4th, 9th& 15th day of treatment. Besides this during this study body weight of rats were recorded on 1st, 4th 9th & 15th day of treatment. On day 16th, blood samples were collected from overnight fasted rats by cardiac puncture. Animals were anaesthetized by mild ether an aesthesia before cardiac puncture. Blood was collected & allowed to stand for one hour, serum was separated by centrifuging & evaluated for different biochemical parameters. Animals were killed & liver, kidney & pancreas were taken out. Histopathology of these organs was also done.

Statistical Analysis

Data obtained from different studies & biochemical estimations is expressed as Mean ± SEM for each group. After this, statistical analysis was carried out using one way analysis of variance (ANOVA) followed by student's t-test. Values $p > 0.05$ were considered non-significant; $p < 0.05$ as significant, $p<0.01$ as highly significant & $p<0.001$ as very highly significant respectively.

Histopathological studies

Pancreas
Kidney
Liver

Organs were taken out, preserved in 10% formalin & sent for histopathological studies.

Physical Characteristics & Percentage Yeild Of Different Extracts

a) Ethanolic extract of *Syzygium cumini* fruits

Weight of dried fruits taken = 500 gms
Weight of extract obtained = 150 gms
% yield = Weight of extract obtained x 100
Weight of dried fruit taken
% age yield of ethanolic extract = 30 %

Extract	Colour	Odour	% Extractive value
50% Ethanolic	Dark Brown	Characteristic	30%

b) Ethanolic extract of *Syzygium cumini* seeds

Weight of dried seeds taken = 1000 gms
Weight of extract obtained =110 gms
% yield = Weight of extract obtained x 100
Weight of dried Seeds taken
% age yield of ethanolic extract = 11%

Extract	Colour	Odour	% Extractive value
50% Ethanolic	Dark Brown	Characteristic	11%

Pharmacological Studies

50% ethanolic extract of *Syzygium cumini* fruits (SGCF) & 50% ethanolic extract of *Syzygium cumini* seeds (SGCS) evaluated for acute oral toxicity studies for 72 hours & sub-acute toxicity studies for 14 days antidiabetic activity,showed following results

Toxicity Studies

Acute Oral Toxicity Tests (72 hour study)

50 % ethanolic concentrate of chose plants, Syzygium cumini natural products (SGCF) & Syzygium cumini seeds (SGCS) were assessed for their intense oral poisonous quality in swiss pale skinned person mice. In main investigation, half ethanolic concentrates of Momordica charantia (PO), in second trial (SGCF) & in third trial (SGCS) were examined for its intense oral danger impacts in swiss pale skinned person mice.

a. at four measurement levels (500, 1000, 1500 & 2000 mg/kg b.w) uncovered accompanying impacts amid intense poisonous quality studies directed in mice for 72 hours. Control mice which had gotten 2% of gum acacia demonstrated ordinary conduct (Table 14*)*

- Prepping: After 48 & 72 hours, no preparing was seen at all four dosage levels.
- Hyperactivity: PO separated at regulated dosage levels had no impact on the action of mice which stayed typical.
- Sedation: After 48 hours, sedation was found in 50 % of creatures at measurements of 500 mg/kg b.w while 100 % of creatures in dosage scope of 1000,1500 & 2000 mg/kg b.w demonstrated sedation ,as creatures tried to avoid panicking & formed with no anxiety.

- Respiratory capture: After 48 hours, 50 % of creatures in measurements scope of 500 mg/kg b.w& 100% creatures in dosage scope of 1000, 1500 & 2000 mg/kg b.w demonstrated respiratory capture showed by bringing of head up in both creatures of these gatherings.
- Writhing : After 48 & 72 hours 50 % of creatures in measurements scope of 500 mg/kg b.w& 100% creatures in dosage scope of 1000,1500 & 2000 mg/kg b.w indicated shakings as tremor in tail & paddling of feet.
- Mortality: 50 % of creatures passed on at dosage of 500 mg/kg b.w following 72 hours while 100 % of creatures in measurements scope of 1000, 1500 & 2000 mg/kg b.w kicked bucket following 24 hours.
- ***Syzygium cumini*** fruit when given at two dose levels (250 & 500mg/kg b.w) to mice of Group IV & Group V respectively also revealed that kidneys do not show any abnormality **(Fig 27a & Fig 27b).**
- **Histopathology Of Liver In Mice**
- **Subacute Toxicity Study (14- Days)**

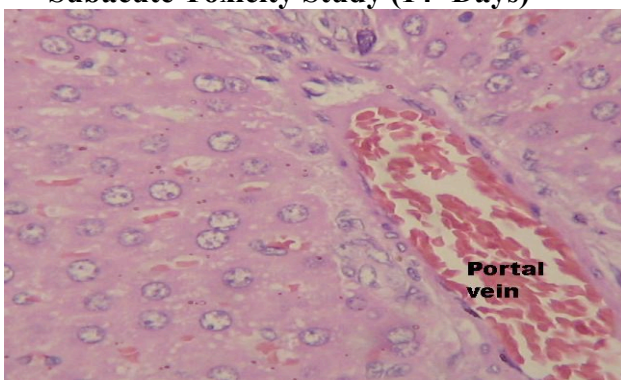

Fig. 1 Group –I –Normal Control, Liver of mice showing normal portal triad area.

Antidiabetic Studies

Antidiabetic studies using alloxan & streptozotocin as diabetic models revealed following results

Alloxan

(10 days study) Effect of various dose of 50% Eth-Extract of *Momordica charantia* whole plant (PO), *Syzygium cumini* fruits (SGCF) &*Syzygium cumini* seeds (SGCS), against alloxan induced diabetes mellitus in rats was studied on following parameters :-

i. **Serum Glucose Levels (Recorded on day 1, day 4, day 7 & day10)**

Ii. **Lipid Profile**

Serum Total Cholesterol Levels

b. Serum Triglycerides Levels

c. Serum HDL Cholesterol Levels

d. Serum LDL Cholesterol Levels

Iii. **Kidney Function Tests**

Serum Urea Levels

b. Serum Creatinine Levels

c. Serum Total Protein Levels

IV Body Weight (Recorded on day 1, day 4, day 7 & day10).

Statistical Analysis

Data obtained from different biochemical estimations is expressed as Mean ± SEM for each group. After this, statistical analysis was carried out using one way analysis of variance (ANOVA) followed by student's t-test. Values $p > 0.05$ were considered non-significant; $p < 0.05$ as significant, $p<0.01$ highly significant & $p<0.001$ very highly significant respectively.

Serum Glucose Levels (mg/dl)

There was very highly significant ($p<0.001$) rise in serum glucose levels in rats in diabetic group (Group II) (271.02±8.18) as compared to levels in normal control group (Group I) (84.71± 6.11 mg/dl). ***Momordica charantia*** extract (100 & 200 mg/kg b.w) showed decrease in serum glucose levels. At dose of 100mg/kg, administered to Group IV, decrease was highly significant ($p<0.01$) (142.82±2.76 mg/dl) while dose of 200mg/kg administered to Group V showed very highly significant decrease ($p<0.001$) (111.58±2.69 mg/dl) in serum glucose levels.

Syzygium cumini fruits at dose of 50 mg/kg administered to rats of Group VI showed highly significant decrease ($p<0.01$) (157.04±3.09 mg/dl), while 100 mg/kg administered to Group VII showed very highly significant decrease ($p<0.001$) (128.64±1.61 mg/dl) & at 200 mg/kg b.w administered to rats of Group VIII also showed very highly significant decrease ($p<0.001$) in serum glucose levels in dose dependent manner (82.82±5.53 mg/dl) as compared to group II that had received only alloxan monohydrate (271.02±8.18mg/dl)($p<0.001$). **Thelevels of serum glucose levels in case of rats administered SGCF at dose of200mg/kg b.w were found to be similar to levels of normal control rats.**

seeds of ***Syzygium cumini*** at dose of 100 mg/kg administered to rats of Group IX showed highly

significant decrease (p<0.01) of (155.54±2.54 mg/dl) while dose of 200 mg/kg b.w administered to rats of Group X also showed very highly significant decrease (p<0.001) (120.74±6.13 mg/dl) in serum glucose levels. Standard anti-diabetic drug, glibenclamide administered to rats of Group III also revealed very highly significant decrease (p<0.001) in serum glucose levels (114.84±3.20 mg/dl) **(Table 28; Fig 28)**

Serum Total Cholesterol levels (mg/ dl)

Syzygium cumini fruits at dose of 50mg/kg administered to rats of Group VI showed highly significant decrease (p< 0.01) of (159.40± 13.65 mg/dl), dose of 100mg/kg administered to rats of Group VII showed very highly significant decrease(p<0.001) (133.17±19.41 mg/dl) while dose of 200 mg/kg administered to rats of Group VIII also showed very highly significant decrease(p<0.001) (98.15±4.78 mg/dl) in total cholesterol levels in dose dependent manner, as compared to group II that had received only alloxan monohydrate. Levels of SGCF at dose of 100 mg/kg & 200mg/kg b.w were found to be very highly significant.

seeds of ***Syzygium cumini*** at dose of 100mg/kg b.w administered to rats of Group IX showed highly significant decrease (p<0.01) (170.55±9.03 mg/dl) while 200 mg/kg b.w administered to rats of Group X also showed very highly significant decrease (p<0.001) in total cholesterol levels (121.68± 14.57 mg/dl). Standard drug, glibenclamide administered to rats of Group III also showed non significant decrease p>0.05 in total cholesterol levels (206.35±6.11 mg/dl) **(Table 30; Fig 29)**

Serum Triglyceride Levels (mg/ dl)

Syzygium cumini when administered to rats showed extremely significant decrease when compared to levels of triglycerides in diabetic control rats of Group II. Rats which received 50 mg/kg administered to rats of Group VI b.w showed highly significant (p<0.01) level of (158.53±13.66 mg/dl) & rats receiving 100 mg/kg b.w administered to rats of Group VII showed very highly significant level (p<0.001) (111.21±12.52 mg/dl) while rats which received 200 mg/kg b.w administered to rats of Group VIII showed very highly significant level (p<0.001) (86.13±11.49 mg/dl)

Moreover, Groups treated with 100 mg /kg b.w of seeds of ***Syzygium cumini*** given to rats of Group IX (127.49±12.66) mg/dl showed highly significant decrease (p<0.01) & 200 mg/kg b.w administered to rats of Group X showed very highly significant (p<0.001) level of (124.29±10.67) of triglycerides. Standard drug glibenclamide administered to rats of Group III showed nonsignificant (p>0.05) level of (184.30±9.68 mg/dl) of serum triglycerides. **(Table 31; Fig 30)**

Serum HDL Cholesterol Levels (mg/ dl)

Syzygium cumini fruits at dose of 50 mg/kg b.w administered to rats of Group VI showed non significant increase(p>0.05) (29.94±4.92 mg/dl) ,while dose of 100 mg/kg b.w administered to rats of Group VII showed significant level (p<0.05) of (30.54±3.23 mg/dl) & dose of 200 mg/kg b.w administered to rats of Group VIII showed significant rise (p<0.05) (32.73±4.04 mg/dl) in serum HDL cholesterol levels in dose dependent manner, as compared to group II that had received only alloxan monohydrate. seeds of ***Syzygium cumini*** seeds at dose of 100mg/kg b.w given to rats of Group IX showed non significant increase (p>0.05) in serum HDL cholesterol levels.(25.04±3.21 mg/dl) & dose of 200 mg/kg b.w showed administered to rats of Group X significant increase (p<0.05) of (31.29±6.12 mg/dl) of HDL Cholesterol was seen. Standard drug, glibenclamide administered to rats of Group III also showed non-significant increase (p>0.05) in serum HDL cholesterol levels. (29.03±3.16 mg/dl) **(Table 32; Fig 31)**

Serum LDL Cholesterol Levels (mg/ dl)

Syzygium cumini fruits in dose levels of 50mg/kg b.w administered to rats of Group VI showed non significant level(p>0.05), of (103.21±10.67 mg/dl), dose of 100 mg/kg b.w administered to rats of Group VII showed non significant level (p>0.05) of (60.54±4.99 mg/dl) & dose of 200 mg/kg b.w administered to rats of Group VIII showed non significant decrease (p>0.05) in LDL cholesterol levels (60.78±6.68) mg/dl in dose dependent manner, as compared to group II that had received only alloxan monohydrate. seeds of ***Syzygium cumini*** at dose levels of 100 mg/kg administered to rats of Group IX (68.91±13.34) mg/dl showed non significant decrease (p>0.05) & at dose of 200 mg/kg bw administered to rats of Group X also showed significant decrease (p<0.05) (50.47±2.63 mg/dl)in serum LDL cholesterol

levels. Standard drug, glibenclamide administered to rats of Group III also showed non-significant decrease ($p>0.05$). In serum LDL cholesterol levels (85.39±7.24 mg/dl) **(Table 33; Fig 32)**

Serum Urea Levels (mg/ dl)

Rats of Group I showed level of serum urea level of (22.32±3.75 mg/dl). Group II rats which received only alloxan monohydrate showed non significant increase ($p>0.05$) in serum urea levels (23.06±0.69 mg/dl).

Dose of 50,100 & 200 mg/kg b.w of fruits of ***Syzygium cumini*** when administered showed non significant changes when compared to activity of diabetic control rats of Group II rats. Dose of 50 mg/kg b.w administered to rats of Group VI showed non-significant level ($p>0.05$), of (26.46±3.84 mg/dl) dose of 100 mg/kg b.w administered to rats of Group VII also showed non significant level ($p>0.05$), of (24.31±2.65 mg/dl). Dose of 200 mg/kg b.w administered to rats of Group VIII also showed non significant level ($p>0.05$) of (17.92±2.44 mg/dl).

Moreover, Groups treated with 100 mg & 200 mg of seeds of ***Syzygium cumini*** seeds also showed non significant change in serum urea levels. Rats of group IX which received 100 mg/kg b.w showed nonsignificant level ($p>0.05$), of (26.11±2.25 mg/dl) & rats of Group X which received 200 mg/kg b.w of ethanolic extract showed non significant level ($p>0.05$), of (29.06± 2.10 mg/dl). Levels in standard drug, glibenclamide administered to rats of Group III showed non-significant level ($p>0.05$) of (20.82± 1.32 mg/dl). **(Table 34; Fig 33)**

Serum Creatinine Levels (mg/ dl)

There was non significant rise ($p>0.05$), in serum creatinine levels in rats in diabetic group (Group II) (1.14±0.12 mg/dl) as compared to normal control (GroupI) (0.85±0.07).

Syzygium cumini fruits in dose levels of 50, 100, & 200 mg/kg b.w showed non-significant changes ($p>0.05$), in serum creatinine levels, as compared to group II that had received only alloxan monohydrate. dose of 50 mg/kg b.w administered to rats of Group VI showed non-significant level ($p>0.05$), of (1.19±0.87 mg/dl), dose of 100 mg/kg b.w administered to rats of Group VII showed level ($p>0.05$), of (1.06±0.64 mg/dl) & dose of 200 mg/kg b.w administered to rats of Group VIII showed non-significant level ($p>0.05$), of (0.89 ±0.65 mg/dl).

Seeds of ***Syzygium cumini*** at dose of 100 mg/kg b.w administered to rats of Group IX showed non significant level ($p>0.05$), of (0.84±0.16 mg/dl) & 200 mg/kg BW administered to rats of Group X (1.02±0.12 mg/dl) also showed non significant changes ($p>0.05$), in serum creatinine levels. Standard drug, glibenclamide also showed non-significant levels ($p>0.05$), in serum creatinine levels (0.76±0.08 mg/dl). **(Table 35; Fig 34)**

Serum Total Proteins Levels (g/ dl)

Syzygium cumini fruits at dose levels of 50, 100, & 200 mg/kg b.w showed non-significant decrease in serum total proteins levels as compared to group II that had received only alloxan monohydrate. dose of 50 mg/kg b.w administered to rats of Group VI showed nonsignificant level ($p>0.05$), of (4.29±1.01 g/dl) & dose of 100 mg/kg b.w administered to rats of Group VII showed non significant level ($p>0.05$) of (4.62±0.99 g/ dl) & dose of 200 mg/kg b.w administered to rats of Group VIII showed non significant level ($p>0.05$) (4.90 ±0.39 g/ dl).

seeds of ***Syzygium cumini*** at dose levels of 100 mg/kg b.w administered to rats of Group IX showed non significant level($p>0.05$) of (3.01±0.70 g/ dl) & 200 mg/kg b.w administered to rats of Group X also showed non significant change($p>0.05$). In serum total proteins levels. (3.36±0.58) g/ dl standard drug, glibenclamide administered to rats of Group III also showed non significant decrease ($p>0.05$) in serum total proteins levels (4.14±0.88 g/ dl). (Besides recording effect on biochemical parameters, effect on body weight of rats revealed following results. **(Table 36; Fig 35)**

Average Body Weight (g)

Dose of 50.100 & 200 mg/kg b.w of fruits of ***Syzygium cumini*** when administered to rats showed extremely significant increase when compared to activity of diabetic control rats of Group II rats. dose of 50 mg/kg b.w administered to rats of Group VI showed highly significant average body weight ($p<0.01$), of (171.47 ±2.74g) & dose of 100 mg/kg b.w administered to rats of Group VII showed very highly significant average body weight ($p<0.001$) of (172.62 ±4.05g) & dose of 200 mg/kg b.w administered to rats of Group VIII

showed very highly significant average body weight ($p<0.001$) of (176.41 ±3.52g)
Moreover, Groups treated with 100 mg/kg b.w administered to rats of Group IX of seeds of ***Syzygium cumini*** showed highly significant increase($p<0.01$) of (162.37±3.08 g) & 200 mg/kg b.w when administered to rats of Group X (167.20 ±3.58g) seeds also showed very highly significant increase ($p<0.001$) in average body weight. Standard drug, glibenclamide administered to rats of Group III showed non significant average body weight ($p>0.05$) of (137.50±3.90g) as compared to rats of group II.

During course of these studies, blood glucose levels & average body weight were observed on day 1, day 4, day, 7 & day 10.

4. Summaryand Conclusion

4.1 Summary

- *Syzygium cumini* fruits was 30%.
- *Syzygium cumini* seeds was 11%.

Preliminary Phytochemical Screening

- *Syzygium cumini* fruits contain alkaloids, glycosides, flavonoids & carbohydrates.
- *Syzygium cumini* seeds contain alkaloids, glycosides & flavonoids.

Toxicity Studies

***a) Acute Oral Toxicity Studies* (72 Hours)**

LD50 of 50% ethanolic extract of

- *Syzygium cumini* fruit was found to be 1000mg/kg b.w.
- *Syzygium cumini* seeds were found to be more than 2000mg/kg b.w.

b) Subacute toxicity study (14 days)

***50% ethanolic extract of Syzygium cumini* fruits showed**

- Significant hypoglycemic effect.
- Non-significant increase in serum urea, creatinine levels & hemoglobin value.
- Non significant increase in liver enzymes like bilirubin levels, total protein levels & albumin levels.
- Significant decrease in SGOT, SGPT, alkaline phosphatase levels & WBC count.
- *Syzygium cumini* fruit also revealed
- No abnormality in liver, normal spleen & kidneys.

Antidiabetic Studies

Alloxan (120 mg/kg b.w) & streptozotocin (50 mg/kg b.w) were found to induce diabetes in rats as evidenced by increased blood glucose levels.

a) Alloxan induced Diabetes mellitus (10 day study)

- **50% ethanolic extract of *Syzygium cumini fruits* (SGCF)**
- Revealed dose dependent **antidiabetic potential** in rats with doses of 50,100 & 200 mg/kg b.w. dose of **200 mg/kg b.w /day** was found to more effective as it decreased blood glucose levels even below standard antidiabetic drug, glibenclamide& values were nearly equal to normal control levels.
- Exhibited significant **hypolipidemic effect** in rats in dose dependent manner.
- **Non significant increase** in kidney function tests, i.e serum urea levels, serum creatinine levels & serum total protein levels.
- Significantly reversed decrease in **body weight** seen in diabetes.

50 % ethanolic extract of *Syzygium cumini seeds* (SGCS)

- Revealed dose dependent **antidiabetic potential** in rats with doses of 100 & 200 mg/kg b.w.
- Exhibited significantly **hypolipidemic** effect in rats.
- Showed **non significant increase** in **kidney function tests,** i.e serum urea levels, serum creatinine levels & serum total protein levels.
- Significantly reversed decrease in **body weight** seen in diabetic rats.
- Glibenclamide, used as standard antidiabetic drug was found to be more effective as it significantly reduced blood glucose levels while it had non significant effect on other parameters.

b) Streptozotocin induced Diabetes mellitus (15 day study)

Histopathological studies of *Portulaca olereacea*

50 % ethanolic extract of *Syzygium cumini fruits* (SGCF)

- Revealed dose dependent **antidiabetic potential** in rats with dose of 50 & 100 mg/kg b.w.
- Exhibited significant **hypolipidemic** effect in rats.
- Exhibited significant **hepatoprotective** effect in rats.

- Significantly reversed decrease in **body weight** seen in diabetes.

Histopathological studies of *Syzygium cumini* fruits

- *Syzygium cumini* fruits when administered (50 & 100 mg/kg b.w) to rats showed islet structure with few inflammatory cells at dose of 50 mg/kg b.w while at dose level of 100mg/kg b.w showed few inflammatory cells in islet of pancreas.
- No abnormality in livers
- No abnormality in kidneys.

50% ethanolic extract of *Syzygium cumini seeds* (SGCS)

- Revealed dose dependent **antidiabetic potential** in rats with doses of 50 & 100 mg/kg b.w.
- Exhibited significant hypolipidemic effect in rats.
- Exhibited significant hepatoprotective effect in rats.
- Significantly reversed decrease in **body weight** seen in diabetes in dose dependent manner

Histopathological studies of Syzygium cumini**seeds**

- *Syzygium cumini* seeds at point when given at dose level of 50 & 100 mg/kg b.w to rats showed islet structure with several inflammatory cells at dose of 50 mg/kg b.w with vacuolation of islet cells while dose of 100 mg/kg b.w to rats showed islet structure with few inflammatory cells.
- No abnormality in liver
- No abnormality in kidney

Glibenclamide, utilized as standard antidiabetic medication was set up to be viable in fundamentally decreasing blood glucose circumstances with non huge impact on different boundaries. It very well may be reasoned that 50 ethanolic passage processing plant, Syzygium cumini natural products and Syzygium cumini seeds have huge antidiabetic possibility. Other than they're set up to have hypolipidemic and Hepatoprotective products as obvious from studies.

4.0 Conclusion

Diabetes mellitus is metabolic issue portrayed by resistance in development of insulin, lacking insulin transmission or both. It is finding opportunity to be champion amongst most extensively seen issue of world. Treatment for diabetes mellitus would be drug that not only controls glycemic level but also prevents development of atherosclerosis & other complications of diabetics. New drugs & new drug delivery systems for insulin have also been introduced. In this research two plants, whole syzygium cumini fruits & Syzygium cumini seeds were evaluated for their toxicity studies & for antidiabetic activity as they have been reported to have hypoglycaemic activity in traditional system of medicine.

Different model systems like alloxan, streptozotocin viruses, & insulin antibodies, hormones like dexamethasone, adrenaline & dithizone are available to screen anti-diabetic activity of given substance In this research chemicals like alloxan & streptozotocin were used to produce marked diabetic effects in animals. Alloxan diabetic model resembles type I diabetes (IDDM) without significant insulin resistance whereas streptozotocin induced diabetic animals inhibit reduced response to insulin in hepatic & peripheral tissues. Further rats treated with streptozotocin display many of features seen in human with uncontrolled diabetes mellitus. In this research, alloxan was given at dose of 120 mg/kg b.w, i.p, while streptozotocin was administered (i.p) at dose of 50 mg/kg, b.w, for inducing diabetes. Albino rats (Wistar strain) of both sexes, weighing 125-250g &swiss albino mice weighing 20-25g, were procured from IIIM Jammu & kept in clean polypropylene cages under uniform conditions of food, water, temperature & degree of nursing care. It was ensured that animals were in good health & free from any infectious diseases. Male & female animals were kept in separate cages so that there was no interference in evaluation of biochemical parameters during period of study. Temperature & humidity of room in which animals were housed were in range of 15-25oC & 70-75 % respectively. In this research, preliminary phytochemical screening, acute oral toxicity study (72 hours), subacute toxicity study (14 days), & antidiabetic studies using alloxan (10 days) & streptozotocin (15 days) for inducing diabetes, were carried out.

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