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Antidiabetic Effect of *Lonicera Ligustrina wall* in Alloxan Induced Diabetes in Wistar Rats

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ABSTRACT

Diabetes, is a group of metabolic disorders characterized by a high blood sugar level (hyperglycaemia) over a prolonged period. The plant extract of *Lonicera Ligustrina wall*. was taken to treat Alloxan induced Diabetes in Wistar rats. *Lonicera ligustrina wall.* is a species of honeysuckle of family Caprifoliaceae. Animals were group into five. The control group animals were given normal saline for 14days. Group II animals were given Alloxan 120mg/kg body weight intraperitoneally until 14th day of the experiment. Group III animals were given Alloxan 120mg/kg i.p and Standard drug metformin hydrochloride 500mg/kg body weight i.p were given until 14 days. Group IV animals were given Alloxan 120mg/kg i.p and test dose of extract *lonicera ligustrina wall* 150mg/kg body weight i.p given up to 14th day. Group V animals were given Alloxan 120mg/kg i.p and test 2 dose of extract of *Lonicera ligustrina wall* 300mg/kg weight i.p given until 14days of the experiment.

Keywords: Hyperglycaemia, *Lonicera ligustrina wall*, Alloxan, Metformin Hydrochloride, Serum parameters.

INTRODUCTION

Diabetes, is a group of metabolic disorders characterized by a high blood sugar level (hyperglycaemia) over a prolonged.Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. Insulin is a hormone which is responsible for helping glucose from food get into cells to be used for energy.Diabetes is a major cause of morbidity and mortality, though these outcomes are not due to the immediate effects of the disorder.

Types of Diabetes

Type 1: Type 1 diabetes accounts for about 5 to 10 percent of cases of diabetes. Most cases of type 1 diabetes develop in children or adolescents, but about 20 percent of new patients are adults. Type 1 diabetes is usually caused by autoimmune destruction of the islets of Langerhans of the pancreas.

Type 2: Type 2 diabetes is far more common than type 1 diabetes. Type 2 diabetes is strongly associated with obesity and is result of insulin resistance and insulin deficiency.

Gestational Diabetes It is a temporary condition associated with pregnancy. In this situation, blood glucose levels increase during pregnancy but usually return to normal after delivery. However, gestational diabetes is recognized as a risk for type 2 diabetes later in life. [3,4]

Lonicera ligustrina wall. is a species of honeysuckle of family Caprifoliaceae, found in Bhutan, India, Nepal, and China. It grows as an evergreen, semi-evergreen, or deciduous shrub approximately 1.5-2.5 meters in height, with leathery or paper-like leaves 0.4-8 × 0.2-1.5 cm in size. Different parts are used such as flower, seed and leaves. *Lonicera ligustrina wall* used in traditional medicine. Used to traditionally for digestive disorders including pain and swelling of the small intestine (enteritis) and dysentery, as laxative, to counteract poisoning, fever, pneumonia and other viral and bacterial infections. Plant extracts demonstrated significant pharmacological activities such as anti-inflammatory, urinary tract disorders, anti-diabetic, anticarcinogenic, to treat respiratory disorders and analgesic etc [1]

METHODS AND MATERIALS

1. Preparation of Plant Extract

The dried leaves of 50g Lonicera *ligustrina wall*. were dried and powdered. 250 ml of alcohol was added to the round bottomed flask. The round bottomed flask was attached to Soxhlet extractor and condenser on a mantle. The leaves powered of lonicera ligustrina wall was loaded into the thimble, which is placed inside the Soxhlet extractor. The solvent was heated using mantle, and as it begins to evaporate, moving to the apparatus to the condenser. The condensate has dripped into the reservoir containing thimble. As the level of solvent reached the siphon it poured back into the flask and the cycle has begun again. The process was continued for a total of 16 hours. As the process finished the Alcohol was been evaporate by direct evaporation method, leaving a small yield of extracted *Lonicera ligustrina* wall. (10-15g) in the round bottom flask.[(2)]

2. Selection of animals

Thirty wistar albino rats of weight 200-250 grams taken, the rats were housed in Polypropylene cages and maintained under standard conditions (12hour day and night cycles, at 25^{o} C and 35-60% humidity). Standardized food pellets feed and tap water were provided.

3. Selection of Doses for the Study

The different doses of alcoholic extract of *lonicera ligustrina wall*. were selected based on the OECD guideline no 420 (acute toxicity studies) and doses were fixed at 150 and 300 mg/kg body weight for 2 different test groups.

4. Chemicals and Dosage

Metformin hydrochloride and Alloxan monohydrate from standard reagents, Hyderabad. All chemicals were of high analytical grade. Alloxan: 120mg/kg (i.p) per body weight, Metformin hydrochloride: 500mg/kg (i.p) per body weight.

Alloxan

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. [(5,6)]

Mechanism of action of alloxan monohydrate

The uptake of alloxan through GLUT2 transporters on the beta cells leads to rapid cell destruction. As alloxan enters the beta cell, reactive oxygen species reduce the alloxan to dialuric acid, which can then go on to reoxidize to alloxan and generate free radicals, such as superoxide. Superoxide can form hydrogen peroxidase, as well as reduce Fe^{3+} ions leading to hydroxyl radicals, both of which damage the DNA of the beta cells leading to fragmentation and ultimate cell death. The hydrogen peroxidase has also been suggested to be involved in causing of intracellular calcium leading to the high initial peak in insulin levels following alloxan administration which can itself cause damage to the beta cells. This cyclical destruction may account for the ongoing action of alloxan long after its clearance from the body with a half-life of only 1.5 min at 37°C.

Toxic effects

Contact with this chemical may cause irritation of the skin and mucous membranes. This chemical induces diabetes mellitus. Symptoms of this disease include hyperglycaemia, hyperlipemia, ketonemia and azoturia. Acute and Chronic hazards: This compound causes irritation to eyes and mucous membranes.

Metformin

Metformin belongs to the biguanide class of antidiabetics with antihyperglycemic activity, that carrying two methyl substituents at position 1. It has a role as a hypoglycaemic agent, a xenobiotic. It derives from a biguanide. It is a conjugate base of a metformin. Metformin is associated with a verylow incidence of lactic acidosis. This agent helps to reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain, and prevents the cardiovascular complications of diabetes. Metformin is not metabolized and is excreted unchanged by the kidneys. [(7,8)]

Mechanism Of Action

Metformin's mechanisms of action are unique from other classes of oral antihyperglycemic drugs. Metformin decreases blood glucose levels by decreasing hepatic glucose production (gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization. It is well established that metformin inhibits mitochondrial complex I activity, and it has generally postulated that its potent antidiabetic effects occur through this mechanism. These processes lead to a decrease in blood glucose, managing type II diabetes and exerting positive effects on glycaemic control.

Therapeutic uses

Metformin has been used in the management of metabolic and reproductive abnormalities associated with polycystic ovary syndrome. However, adequate and well-controlled clinical trials evaluating metformin therapy for polycystic ovary syndrome remain limited, particularly regarding long-term efficacy, and available data are conflicting regarding the benefits of the drug in ameliorating various manifestations of the condition. Metformin Hydrochloride tablets, USP are indicated as an adjunct to diet and exercise to improve glycaemic control in adults and children with type 2 diabetes mellitus

5. Preliminary phytochemical screening methods [(9)]

The Phytochemical constituent tests were carried out for the given plant *lonicera ligustrina wall*. extract using standard procedures by using Pharmacognosy book by C.K. Kokate to identify the constituents

Test for carbohydrates

Molisch's Test: Molisch's test is a general test for carbohydrates. This test is given by almost all of the carbohydrates. In this test, concentrated sulfuric acid converts the given carbohydrate into furfural or its derivatives, which react with α-naphthol to form a purple-coloured product

Fehling's Test: This test is given by reducing sugars. To the aqueous solution of carbohydrate Fehling's solution is added and heated in water bath. The formation of red precipitate confirms the presence of reducing sugars. The copper ions present in Fehling's solution in +3 state is reduced to +2 oxidation state and in alkaline medium it is precipitated as red cuprous oxide.

Test for Amino acids

Ninhydrin test: To the 1ml of amino acid solution in a test tube, add 1 drop of the ninhydrin reagent. Keep it in boiling water bath and observe the formation of purple colour.

Test for Alkaloids

Mayer's test: To about 3 ml. of extract, a few drops of Mayer's reagent are added. Precipitate is formed.

Dragendorff's test: To about 3 ml of extract, a few drops of Dragendorff's reagent are added. Brownish fluorescent precipitate is formed.

Wagner's test: To about 3 ml of sample solution, a few drops of Wagner's reagent are added. Brownish precipitate is formed.

Hager's test: To 2-3 ml of filtrate with few drops of Hager's reagent gives yellow precipitate.

Test for Tannins

Ferric chloride test: Tannin extract + Ferric Chloride Solution, Gives Blue colour: Hydrolysable tannin, Brownish green colour: Condensed tannin

Match stick test (Catechin test): Match stick dipped in aqueous plant extract, dried near the flame, Moistened with conc. HCl, Warm near flame. Match stick wood turns Pink-Red colour (Phloroglucinol formation)

Test for terpenoids

Salkowski reaction: To 2ml of extract, add 2ml of chloroform and 2ml of conc. H_2SO_4 solution. Shake well, Chloroform Layer appears red and acid layer appears greenish yellow fluorescent.

Liebermann Burchard reaction: Mix 2ml of extract with chloroform, add 1-2ml acidic anhydrate and 2 drops of can H_2SO_4 from the side of the test tube then blue, then green colour appears

Test for glycosides

Keller-kiliani test: To the alcoholic extract of drug equal volume of water and 0.5 ml of strong lead acetate solution was added, Shaked and filtered. Filtrate was extracted with equal volume of chloroform. Chloroform extract was evaporated to dryness and residue was dissolved in 3 ml of glacial acetic acid followed by addition of few drops of $FeCl_3$ solution. The resultant solution was transferred to a test tube containing 2 ml of conc. H_2SO_4. Reddish brown layer is formed, which turns bluish green after standing due to presence of digitoxose.

Baljet test:thick section of leaf of digitalis or the part of drug containing cardiac glycoside, when dipped in sodium picrate solution, it forms yellow to orange colour in presence of aglycones or glycosides.

Test for saponins

Foam test: To 1 gm of drug add 10 – 20 ml of water, shake for few minutes, formation frothing which persists for 60–120 s in presence of saponins.

Tests for Flavonoids

Ammonia test: Filter paper dipped in alcoholic solution of drug was exposed to ammonia vapor. Formation of yellow spot-on filter paper.

Shinoda test: To the alcoholic extract of drug magnesium turning and dil. HCl was added, formation of red colour indicates the presence of flavonoids. To the alcoholic extract of drug zinc turning and dil. HCl was added, formation of deep red to magenta colour indicates the presence of flavonoids

Test for proteins

Ninhydrin test: To the test solution add a 1/4th volume of Ninhydrin solution and boil and cool. Formation of a blue colour indicates the presence of protein.

Xanthohydrin test: Mix the sample solution with concentrated nitric acid, boil and cool. Then add 40% sodium hydroxide drops. Formation of orange colour solution indicates protein.

6. Experimental Design and Procedure

Design

Thirty Wistar Albino rats of weight 200-250g were selected for the study. Animals were divided into five groups of each six animals.

Group 1: Control group received normal saline

Group 2: Negative control group (Alloxan induced group received alloxan 120mg/kg)

Group 3: Standard group (received Alloxan + Metformin hydrochloride 500mg/kg)

Group 4: Treatment 1 group (received Alloxan+ alcoholic extract of *lonicera ligustrina wall* 150mg/kg)

Group 5: Treatment 2 group (received Alloxan+ alcoholic extract of *lonicera ligustrina wall* 300mg/kg)

Treatment schedule

Animals were group into five groups as explained above. The control group animals were given normal saline for 14days. Group II animals were given Alloxan 120mg/kg body weight intraperitoneally until 14th day of the experiment. Group III animals were given Alloxan 120mg/kg i.p and Standard drug metformin hydrochloride 500mg/kg body weight i.p were given until 14 days. Group IV animals were given Alloxan 120mg/kg i.p and test dose of extract *lonicera ligustrina wall* 150mg/kg body weight i.p given up to 14th day. Group V animals were given Alloxan 120mg/kg i.p and test 2 dose of extract of *Lonicera ligustrina wall* 300mg/kg weight i.p given until 14days of the experiment. On 15th day of the experiment Blood glucose levels was determined by using Gluco meter.

Serum sample preparations

The animals were sacrificed on 15th day using anaesthesia, blood was collected by tail vein method. Blood was centrifuged using Remi cool centrifuge at 4000rpm for 15 mins. Serum was separated for the estimation of various biochemical parameters like Serum Cholesterol, Serum Urea, Creatinine, Triglycerides, HDL, LDL, Alkaline phosphatase, SGPT and SGOT

HISTOPATHOLOGY EXAMINATION

The animals were sacrificed on 15th day by CO_2 inhalation through euthanasia chamber. later pancreases were exceeded out after the experimentation and fixed in formalin (10%v/v). the tissue was processed and secretions were cut. The slides were prepared and stained with haematoxylin and Eosin and examined under microscope (100x) and photomicrographic were taken.

STATISTICAL ANALYSIS

Significant effects of groups were observed on Serum creatinine level ($p<0.0001$), urea level($p<0.0001$), alkaline phosphatase level (0.0001), Total cholesterol, LDL and HDL cholesterol level (0.0009). Serum parameters levels of test group and standard group are increased compared to negative control group.

RESULTS

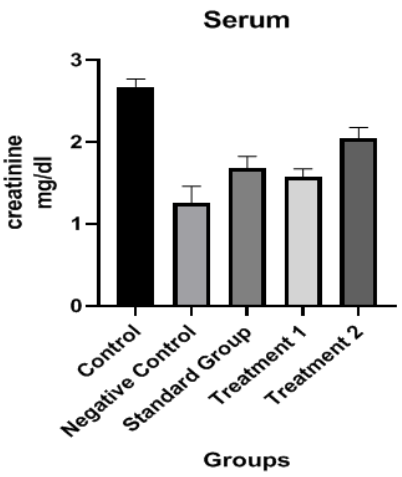
Table 1: Preliminary phytochemical analysis of *Lonicera ligustrina wall*

S.NO	PHYTOCHEMICALS	*LONICERA LIGUSTRINA WALL.*
1.	Alkaloids	+
2.	Phenolic compounds	-
3.	Tannins	+
4.	Glycosides	+
5.	Flavonoids	+
6.	Lipids	-
7.	Saponins	+
8.	Amino acids	-
9.	Terpenoids	+
10.	Steroids	-
11.	Carbohydrates	+

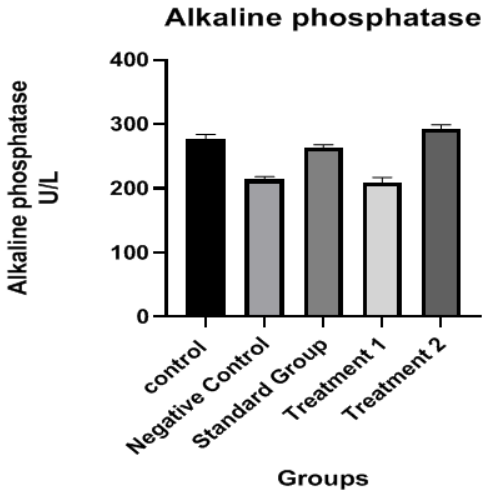
Serum Parameters

Following the Serum parameter levels test, 4-5ml of blood was collected from each rat via cardiac puncture. Serum parameters such as creatinine, urea, alkaline phosphatase, cholesterol, LDL and HDL cholesterol and triglycerides were examined at S.V. Diagnostic Centre, Gandimaisamma, Hyderabad, Telangana (India).

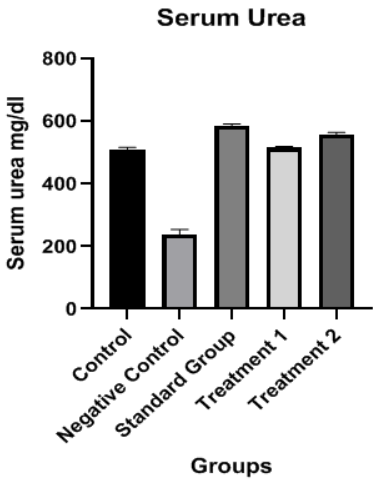
Graph 1,2,3,4,5,6 and 7 shows different serum parameters i.e., Creatinine, Urea, Alkaline phosphatase, Cholesterol, LDL & HDL cholesterol and Triglycerides respectively.



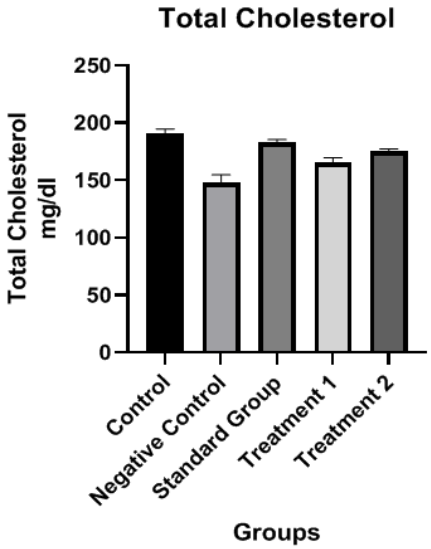
Graph 1: Serum Creatinine



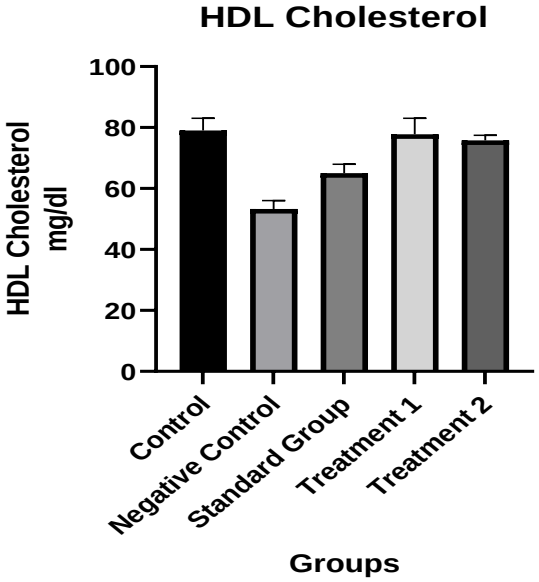
Graph 2: Serum Alkaline Phosphatase



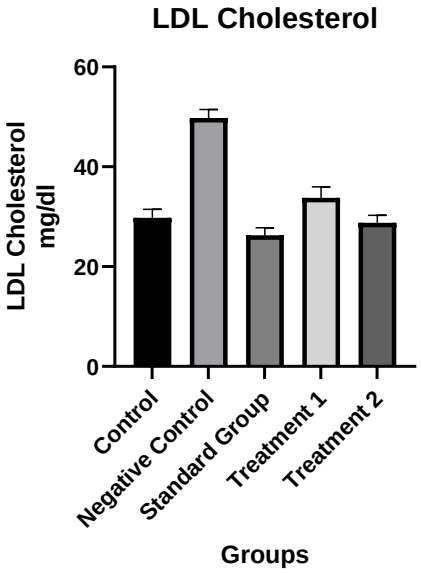
Graph 3: Serum Urea



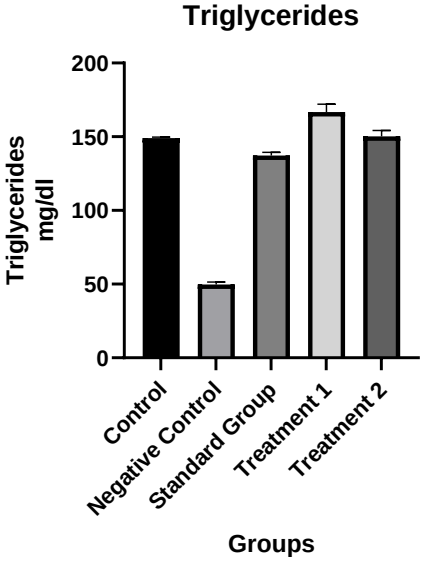
Graph 4: Total Cholesterol



Graph 5: HDL Cholesterol



Graph 6: LDL Cholesterol



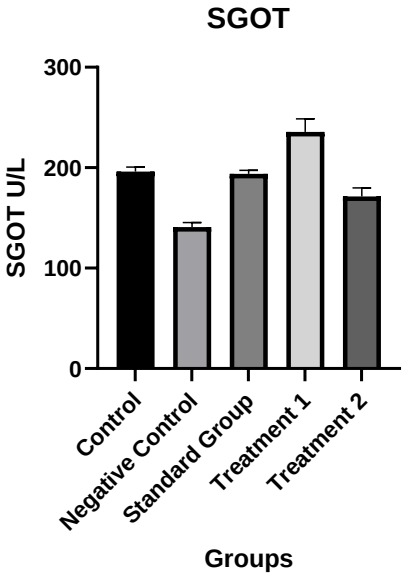
Graph 7: Triglycerides

Effects on Antioxidant parameters

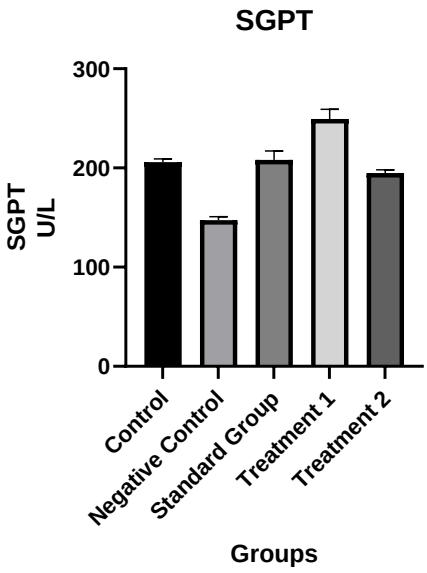
Significant effects on antioxidant parameters such as SGOT & SGPT. SGOT($p<0.0001$) and SGPT($p<0.0001$). SGPT and SGOT levels of test group is increased compared to standard group and also levels of negative control group decreased compared to test and standard group.

Graph 8 and 9 shows the levels of SGOT & SGPT respectively.

Control	Negative Control	Standard Group	Treatment 1	Treatment 2
190	146	198	220	160
200	135	190	230	170
199	142	192	242	175
195	140	195	250	180



Graph 8: SGOT level



Graph 9: SGPT level

SGPT

Control	Negative Control	Standard Group	Treatment 1	Treatment 2
206	150	200	242	190
210	149	202	240	195
205	148	210	260	198
202	142	220	255	196

Histopathological Results

1. Control Group

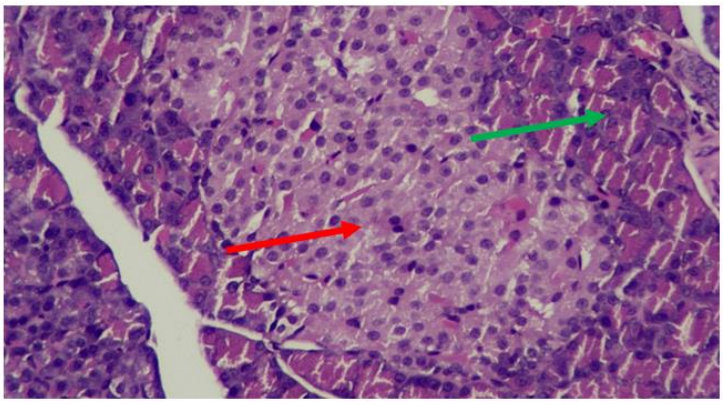


Fig 1: Control group histopathological structure

Normal morphology of beta cells of islets of pancreas- red arrow and normal morphology of acinar cells of pancreas – green arrow

2. Negative Control Group

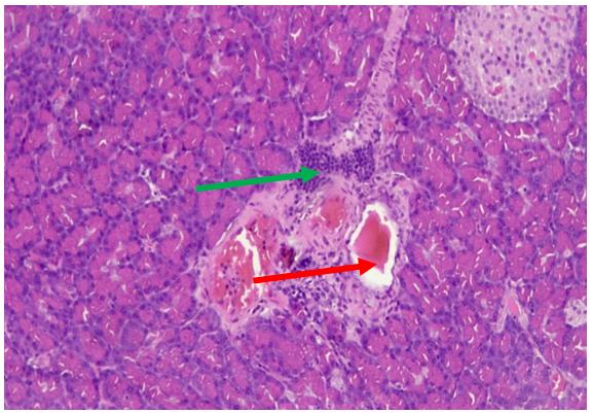


Fig 2: Negative control group histopathological structure

Complete atrophy and fibrosis and inflammation of islets of pancreas with infiltration of inflammatory cells – red arrow andfibrosis [green arrow].

3. Standard Group

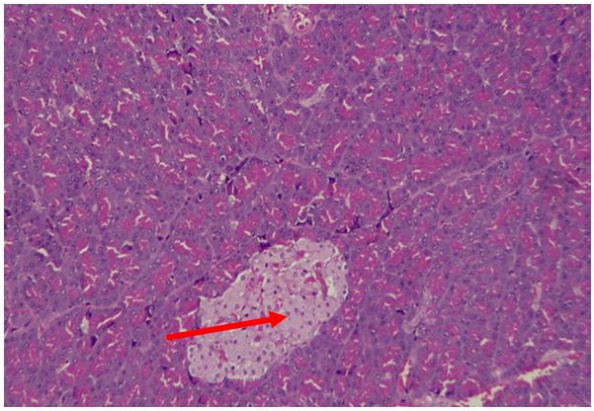


Fig 3: Standard group histopathological structure

Mild hemorrhages were observed in beta cells in islets of pancreas - arrow

4. Treatment 1 and 2 group

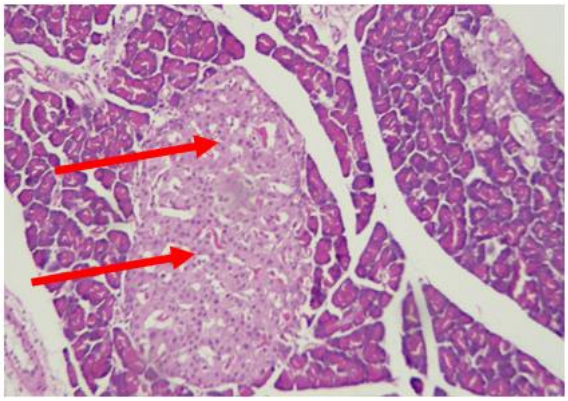


Fig 4: Treatment 1 histopathological structure

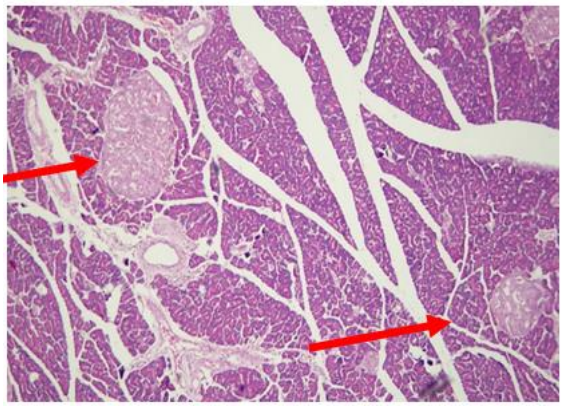


Fig 5: Treatment 2 Histopathological structure

Moderate hypertrophy of islets of pancreas with hyperplasia of beta cells and mild degenerative changes in beta cells of pancreas – arrow

Treatment 2 Group:

Moderate hypertrophy of islets of pancreas with hyperplasia of beta cells and mild degenerative changes in beta cells of pancreas – arrow

DISCUSSION & CONCLUSION

Alcoholic extractof *Lonicera ligustrina wall* contains following Phytochemicals which are concluded by testing the extract with Preliminary phytochemical screening methods, they are alkaloids, tannins, carbohydrates, terpenoids, flavonoids, saponins, glycosides. In this, flavonoids and saponins are responsible for antidiabetic activity.

The above Serum parameters such as Serum Creatinine, Serum Urea, Serum Alkaline phosphatase, Serum Cholesterol, LDL cholesterol, HDL Cholesterol and Triglycerides serum levels of Treatment group animals with Standard group animals, control group animals are compared with treatment group and standard group, and also negative control group animals are compared with standard group and treatment group. Serum levels of test group increased compared to standard and negative control group. It was concluded that plant showing Antidiabetic activity.

Also, histopathological studies also done and the above histopathological studies shows mild to moderate hypertrophy of islets of pancreas with hyperplasia of beta cells and mild degenerative changes in beta cells of pancreas compared to negative control group whereas in negative control group complete atrophy was observed.

Although the impact of traditional drug was controversial, there are hopes for its effectiveness. Much research has been performed on lonicera species i.e., *Lonicera japonica, Lonicera nitada*, etc but *lonicera ligustrina*, some research has been performed. The present study aimed to perform the antidiabetic effect of *Lonicera ligustrina* on alloxan induced diabetes on wistar rats was studied. It indicated an effective activity on diabetes and associated symptoms.

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