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EVALUATION OF PROTECTIVE EFFECT OF THE POLYPHENOLS OF RED MARINE ALGA *SYMPHYOCLADIA LATIUSCULA* AGAINST POLOXAMER-407 AND FREE FATTY ACID-INDUCED PANCREAS DYSFUNCTION

ZIONMIT LEPCHA*, DEEPAK KUMAR JHA[1]
Department of Pharmacology, Karnataka College of Pharmacy, Karnataka, India
*Corresponding author's email id.: lepchazionmit@gmail.com

ABSTRACT

Background: Natural products have played a huge role in the evolution of pharmaceuticals and agrichemicals which has helped amplify the welfare of living beings since centuries. Marine Kingdom is distinguished in terms of potential organisms with profuse sources of vast and diverse biologically potent compounds. *Symphyocladia latiuscula* (Yamada), a red marine alga belonging to the family Rhodomelaceae, from the order Ceramiales, found in the banks of North China, Japan and Korea has shown promising therapeutic potential in the treatment of various diseases. **Objectives:** In this study, investigation of the protective potential of the polyphenols of red marine alga *Symphyocladia latiuscula* (SL) in rats with pancreatic dysfunction induced by poloxamer-407 and free fatty acid was carried out. **Methods:** Normal control group received vehicle orally (p.o), daily, the disease group received co-administration of Poloxamer-407 (10 mg/kg) i.p and FFA (palmitic acid - 200 mg/kg, twice a day, i.p) for 5 weeks, daily to induce pancreatic dysfunction, the treatment group received disease control + *Symphyocladia latiuscula* polyphenols (200 mg/kg) p.o for 4 weeks and the standard group received disease control + metformin (100 mg/kg) p.o for 4 weeks. The blood glucose was monitored biweekly. Protective effects of *Symphyocladia latiuscula* on Poloxamer-407 and P.A induced pancreas dysfunction in rats were determined by various in vivo and in vitro models - Fasting and post-prandial glucose, Sucrose-preference test, Glucose tolerance test, Insulin tolerance test, Insulin Assay, Homeostasis Model of Insulin Resistance (HOMA-IR), biochemical parameters of serum, various antioxidant enzymes, and histopathological damages in pancreas. **Results:** The *S. latiuscula* exhibited significant reduction in fasting and post prandial blood glucose, sucrose preference, total cholesterol, triglycerides, LDL, VLDL, HbA1c, Superoxide dismutase, Malondialdehyde, Nitric oxide, improvement of insulin production, glucose tolerance, insulin tolerance, HOMA-IR; increase in HDL and glutathione levels in the treated group and amelioration of histological changes of test groups as well as standard group as compared to the disease group. **Conclusion:** *Symphyocladia latiuscula* treatment showed a protective and comparable effect with metformin treatment in pancreas dysfunction rat model.

Keywords: *Symphyocladia latiuscula*, pancreatic dysfunction

INTRODUCTION

Diabetes, a chronic degenerative disease, is a detrimental epidemic of the 21st century. It results in long-term complications affecting the autonomic and peripheral nervous systems, eyes, kidneys, etc.[1] Diabetes Mellitus is a metabolic disorder distinguished by persistent hyperglycemia with disturbance of protein, carbohydrate or fat metabolism due to deficiency in insulin action, insulin secretion, or both (WHO, 1999). The Diabetic population in the world is expected to grow about 366 million worldwide by 2030 despite the availability of different synthetic medication and insulin therapy.[2] It accounts for more cases of amputations, loss of vision, end-stage kidney disease, than any other disease in adults.[3] A pathophysiology of type 2 diabetes, insulin resistance, is correlated with obesity - lipotoxicity in obesity causes the death and dysfunction of pancreatic β-cells and deficient insulin production, causing type 2 diabetes, leading to pancreatic dysfunction.[4]

The after effects associated with treatment available presently – synthetic drugs, is an alarming concern on long term basis.[2] Natural products have played a huge role in the evolution of pharmaceuticals and agrichemicals which has helped amplify the welfare of living beings since centuries.[5] Marine Kingdom is distinguished in terms of potential organisms with profuse sources of vast and diverse biologically potent compounds. Over the last few decades, several novel compounds indicating striking bioactive potential have been isolated from the marine organisms. Algae are simple life forms consisting of chlorophyll and either composed of one or a group of cells as colonies. While compared to other algal groups, red algae possesses the most bioactive potential.[6] *Symphyocladia latiuscula* (Yamada) belongs to the family Rhodomelaceae, from the order Ceramiales, found in the banks of North China, Japan and Korea. The specific chemical constituent, bromophenols of the alga has shown positive therapeutic potential in the treatment of various diseases. Bromophenols isolated from *Symphyocladia latiuscula* has been distinguished as the main component responsible for various pharmacological activities like peroxynitrite scavenging,[7] aldose reductase inhibition,[8] antifungal action,[9] antiviral,[10] cytoprotective,[7] Taq DNA polymerase inhibition,[11] anti-proliferators,[12] anti-bacterial and anti-inflammatory actions.[13] This study aimed to investigate the protective effects of *Symphyocladia latiuscula* algae extract against Fatty Acid (Palmitic acid) and Poloxamer-407 induced pancreas dysfunction in rats.

Increased levels of free fatty acid (FFA) like palmitate (PA), have been considered as the cause of β-cell mass insufficiency in pancreas and β-cell dysfunction since long, called as lipotoxicity and leading to the development of type 2 diabetes mellitus in due course. Free fatty acid increases the endoplasmic reticulum (ER) stress in pancreatic β-cells, leading to abnormally regulated protein folding, trafficking, processing, and calcium buffering. When exposed to saturated FFAs such as Palmitic acid, a sustained ER Ca2+ depletion occurs, accompanied by mitochondria dysfunction and nitric oxide (NO) free radicals production through the increased activity of inducible nitric oxide synthase (iNOS), aggravating β-cell apoptosis.[14] Poloxamer-407 (PX-407) is a non-ionic copolymer commonly used in pharmaceutical formulations such as surfactants, solubilizing agents, emulsifying agents and in vivo as absorbance enhancers. Intraperitoneal administration of PX-407 in rats results in hyperglycemia with low degree of toxicity (LD50 ≥ 1.8 g/kg body weight) and causes impaired response in the glucose tolerance test leading to loss of b-cell sensitivity to glucose. Therefore, Poloxamer-407 is considered as a convenient model for the experimental study of the activity of antidiabetic agents.[15]

The present study was conducted to investigate and compare the possible protective of the methanolic extract of *Symphyocladia latiuscula* versus metformin against Palmitic acid and Poloxamer-407 induced Pancreatic dysfunction in rats. Metformin, a commonly used biguanide was used as a reference anti-diabetic drug.[16]

MATERIALS AND METHODS

Plant material and extraction

The marine red microalga *Symphyocladia latiuscula* was procured from the South China sea coast through a reputed commercial dealer. The authentication of the sample was carried out by Amir Chem Pvt. Ltd, Pithampur, M.P (Batch No. – ACPL/CSH07/141101). Dried, fine powders of SL were subjected to continuous hot extraction with 70% methanol for 3 h with reflux at 70–75 °C three times successively. The extract was concentrated to half its volume and partitioned with n-hexane (five times) to remove pigments & lipids. Aqueous fraction contained soluble polyphenols (positive with Folin-Ciocaulteu's phenol reagent) that were precipitated with acetonitrile (1:1), concentrated in a rotary evaporator and lyophilized to obtain light brown crystals. The polyphenol fraction was designated *Symphyocladia latiuscula* polyphenols (SLPP).[17]

Animals

24 Wistar Albino rats weighing 150-180 gm were maintained in standard laboratory conditions at room temperature (25±2 °C) with a 12-hr light/12-hr dark cycle free access to diet and water. The study protocol was duly approved by the Institutional Animal Ethics Committee at Karnataka College of Pharmacy, Bangalore (Reg No: 1564/PO/Re/S/11/CPCSEA).

Acute Oral Toxicity

Acute oral toxicity was carried out following OECD-423 guidelines in Female Albino Wistar rats. The rats were fasted overnight on water ad libitum diet. 5 mg/kg of poly phenols was administered as the starting dose to three animals in each group orally. The dose was assigned as a toxic dose if the mortality was observed in two or three animals. If mortality was observed in one animal, the same dose was administered again to verify the toxic dose in three animals. The method was repeated for higher doses - 50, 300, and 2000 mg/kg body weight if mortality was not observed. Animals were observed individually after dosing at least once during the first 30 minutes and periodically during the first 24 hours. During the first 4 hours, special attention was given to the animals and daily thereafter, for a total of 14 days.

Induction of Pancreas Dysfunction

Diabetes was induced by co-administration of free fatty acid (palmitic acid, 200 mg/kg)[18] twice a day intraperitoneally at an interval of 5 hours and Poloxamer-407 (10 mg/kg)[19] intraperitoneally once a day for 5 weeks. Blood glucose was monitored biweekly.

Experimental Design

Animals were divided into four groups: normal control (group I), disease control (group II), test (group III) standard (group IV). The experimental procedure is summarized in Table 1.

Sl. No.	Group	Treatment	Duration
I	Normal Control	Vehicle p.o daily	--
II	Disease Control	co-administration with Poloxamer-407 (10 mg/kg) i.p and FFA (palmitic acid - 200 mg/kg, twice a day, i.p)	5 weeks
III	Test Group	Disease control + *Symphyocladia latiuscula* polyphenols (200 mg/kg) p.o.	4 weeks
IV	Standard Group	Disease control + metformin (100 mg/kg) p.o.	4 weeks

Table 1: Experimental Protocol.

Sample Collection

Under mild pentobarbital anesthesia, blood samples were collected at the end of the treatment by cardiac puncture. Each animal was then sacrificed with a high dose of pentobarbital; pancreas was isolated and divided into two portions; one portion was homogenized to be used for biochemical analysis; the other portion was preserved in 10% formaldehyde for histological examination.

Fasting and post-prandial glucose

Blood samples were collected from the tail vein of overnight fasted rats the day before sacrifice. The rats were then given glucose solution (3 g/kg b.w.) and blood samples was collected after 2 hours. Blood glucose was measured using a digital glucometer (Accu-Chek).

Sucrose-preference test

All rats were subjected to sucrose preference test to evaluate the handling stress. After the last injection, one bottle filled with water and the other bottle containing 2% sucrose were both placed in the cage around 6pm. Next day, we measured the weight of these two bottles. Sucrose preference was reported as the difference between total sucrose consumption divided by total liquid consumption.[20]

Glucose tolerance test

Food was removed, and after 12 h of fasting, the experiments were performed. Blood glucose was measured at time interval of 0, 15, 30, 60, 90, and 120 min after i.p injection of 40% sterile glucose solution dissolved in saline at a dose of 1.5 g/kg. Using a glucometer, blood glucose was measured by the blood sample collected from the tail tip of the rats.[18]

Insulin tolerance test

Mice fasted for 4 h was given insulin (0.75 U/kg) i.p, and tail was bled for glucose measurement at 0, 15, 30, 60, 90 and 120 min.[18]

Insulin assay

Insulin in serum samples was estimated by ELISA (Insulin ELISA kit, Mercodia, Sweden) as per the manufacturer's manual.

Determination of Homeostasis Model of Insulin Resistance (HOMA-IR)

Insulin resistance was evaluated by HOMA-IR as follows:

HOMA-IR = [Fasting insulin (µU/ml) x Fasting glucose (mmol/L)] /22.5

An increase in HOMA-IR value indicates insulin resistance.

Biochemical parameters

Total Cholesterol, Triglycerides, HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (very low-density lipoprotein) was estimated by enzymatic method using Monozyme diagnostic kit.

Determination of HbA1c level

HbA1c level was estimated according to the method of Bannon using a commercial diagnostic kit.[24]

Assay of oxidative stress and antioxidant defense system parameters:

Malondialdehyde (MDA)

The pancreatic tissue was homogenized in trichloroacetic acid (TCA) and the homogenate was used to estimate malondialdehyde (MDA). Briefly, lipid peroxidation was induced by adding ferric chloride (10 µl, 400 mM) and l-ascorbic acid (10 µl, 400 mM) to a mixture containing pancreas homogenate (0.3 ml) in phosphate buffer solution (5 ml, pH 7.4, 0.2 M). After incubation for 1 hr. at 37°C, the reaction was stopped by adding HCl (2 ml, 0.25 N) containing TCA (1 ml, 15% w/v) and TBA (0.5 ml, 0.375% w/v) boiled for 15 min, cooled, and centrifuged, and the absorbance of the supernatant was measured at 532 nm. The results were expressed as nmol MDA/g protein.[25]

Nitric oxide (NO)

Pancreas NO level was evaluated indirectly via measuring NO metabolites by nitrate/nitrite colometric assay kit. 100 µL of the analysed sample was added to 600 µL of the Griess reagent (equi-volumes of 2% sulfanilamide in 2.5% phosphoric acid and 0.1% w/v N'1-(1-naphthyl)-N-2-diethylethylenediamine in distilled water were mixed just before use), the mixture was mixed and after 10 min, the absorbance at 540 nm was measured. NO contents were expressed as µM/g tissue protein.[26]

Serum superoxide dismutase (SOD):

To 2.78 mL sodium carbonate buffer (0.05 mM, pH 10.2), 100 µL of 1 mM EDTA and 20 µL tissue supernatant were added and incubated at 30°C for 45 min. The reaction was initiated by adding 100 µL of adrenaline. The change in the absorbance was recorded at 480 nm for 3 min. Sucrose was used as a blank. The values were expressed as units/min/mg of protein.[27]

Determination of Reduced Glutathione (GSH):

GSH was determined utilizing Ellman reagent.[28,29] Equal volumes of pancreatic homogenate and 10% trichloroacetic acid (TCA) were mixed and centrifuged at 750 g for 5 mins. 0.1 ml of supernatant was mixed with 1.7 ml of 0.1 M potassium phosphate buffer pH 8. Then 0.1 Ellman's reagent was added. The optical density was measured at 412 nm against blank. The results are expressed as nmol/min/mg of protein.

Statistical analysis

The results are expressed as Mean ± S.E.M. from n=6 rats in each group. Data were analyzed using statistical software Graph Pad Prism version 9. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test compared between Normal control (Untreated) vs all groups.

RESULTS

Effect on Fasting and Post-prandial glucose

Induction of Palmitic acid and Poloxamer-407 resulted in a significant raise in fasting glucose levels in disease control group (204.5 ± 1.478 mg/dL; $p < 0.001$) compared to normal control (untreated) (83.33 ± 1.476 mg/dL). Test group (*Symphyocladia latiuscula* (SL) treated) (127.2 ± 2.301mg/dL; $p < 0.001$) and standard group (94.50 ± 1.478mg/dL; $p < 0.001$) showed significantly reduced levels compared to disease control group. Fig 1.

Induction of Palmitic acid and Poloxamer-407 resulted in a significant raise in post prandial glucose levels in disease control group (355.3±2.951mg/dL; $p < 0.001$) compared to normal control (untreated) (121.0±1.155mg/dL). SL treated group (167.0± 2.781mg/dL; $p < 0.001$) and standard group (152.0± 1.789mg/dL; $p < 0.001$) showed significantly reduced levels compared to disease control. Fig 2.

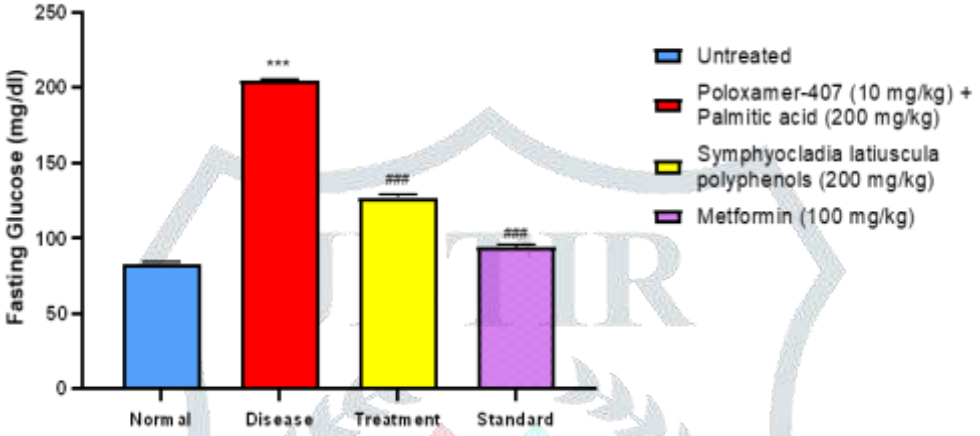


Fig 1 Fasting Glucose
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

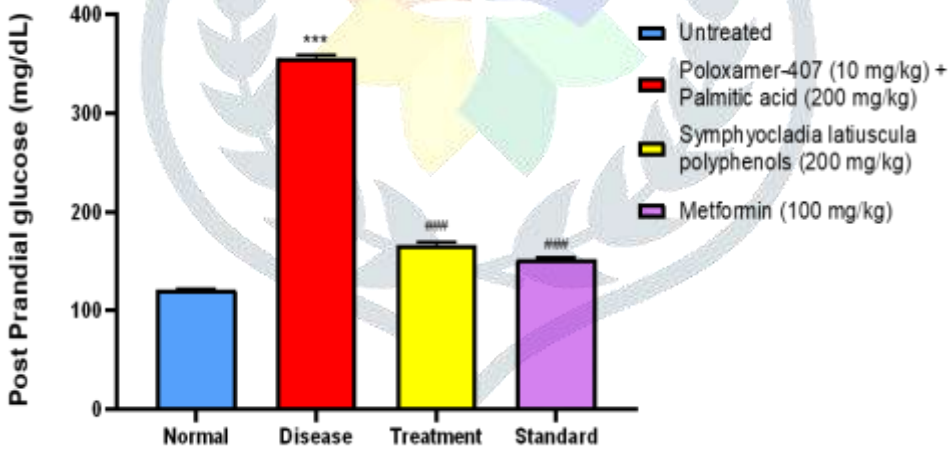


Fig 2 Post Prandial Glucose
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

Sucrose-preference test

Induction with Palmitic Acid and Poloxamer-407 resulted in a slight raise in sucrose preference in disease control group (71.72±0.5735 %) compared to normal control (untreated) (56.57±1.114 %). SL treated group (66.76±0.6502 %) and standard group (62.01±0.3640 %) showed slightly reduced levels compared to disease control. However, the p value was non-significant.

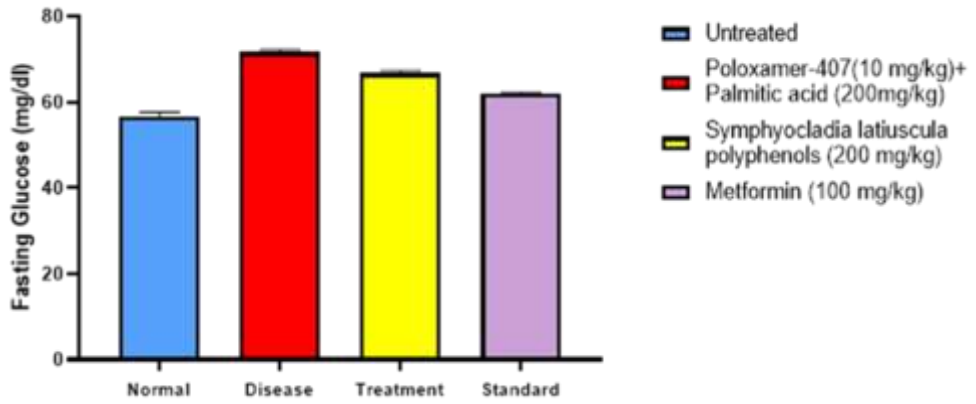


Fig 3 Sucrose Preference test

Glucose Tolerance test

Induction with Palmitic Acid and Poloxamer-407 resulted in dose dependent deterioration in glucose tolerance in disease control group ($^{*}p<0.05$) compared to normal control (untreated). SL treated group ($^{\#}p<0.05$) and standard group ($^{\#}p<0.05$) showed dose dependent improvement compared to disease control.

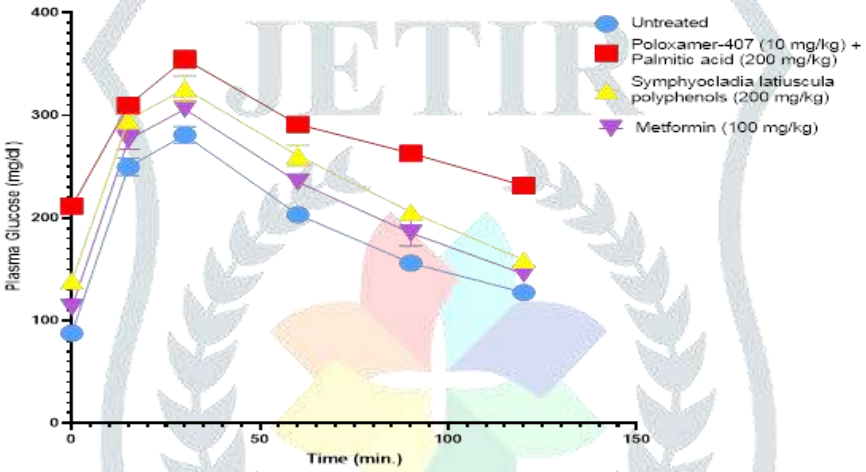


Fig 4 Glucose Tolerance test
* ($p < 0.05$) versus normal control
$^{\#}$ ($p < 0.05$) versus disease control

Glucose Tolerance test

Induction with Palmitic Acid and Poloxamer-407 resulted in dose dependent deterioration in insulin tolerance in disease control group (*p<0.05) compared to normal control (untreated). SL treated group ($^{\#}p<0.05$) and standard group ($^{\#}p<0.05$) showed dose dependent improvement compared to disease control.

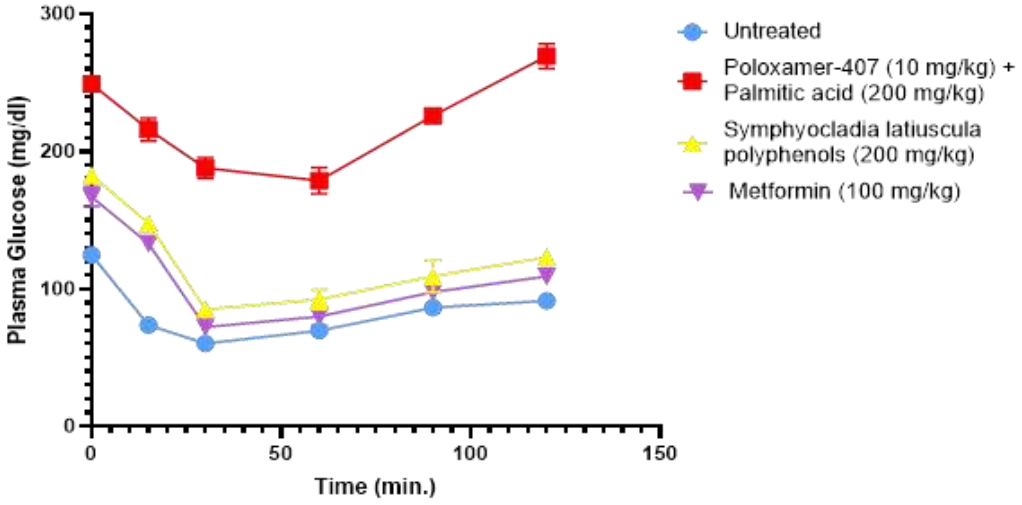


Fig 5 Insulin Tolerance test
* ($p < 0.05$) versus normal control
# ($p < 0.05$) versus disease control

Homeostasis Model of Insulin Resistance (HOMA-IR)

A significant reduction in HOMA-IR index was observed (3.304±0.1973 mIU/ml*mmol/L; $p < 0.001$) compared to normal control (untreated) (5.885±0.02927 mIU/ml*mmol/L). SL treated group (4.081±0.02611 mIU/ml*mmol/L; $p < 0.01$) and standard group (4.916±0.02975 mIU/ml*mmol/L; $p < 0.001$) showed significantly reduced levels compared to disease control.

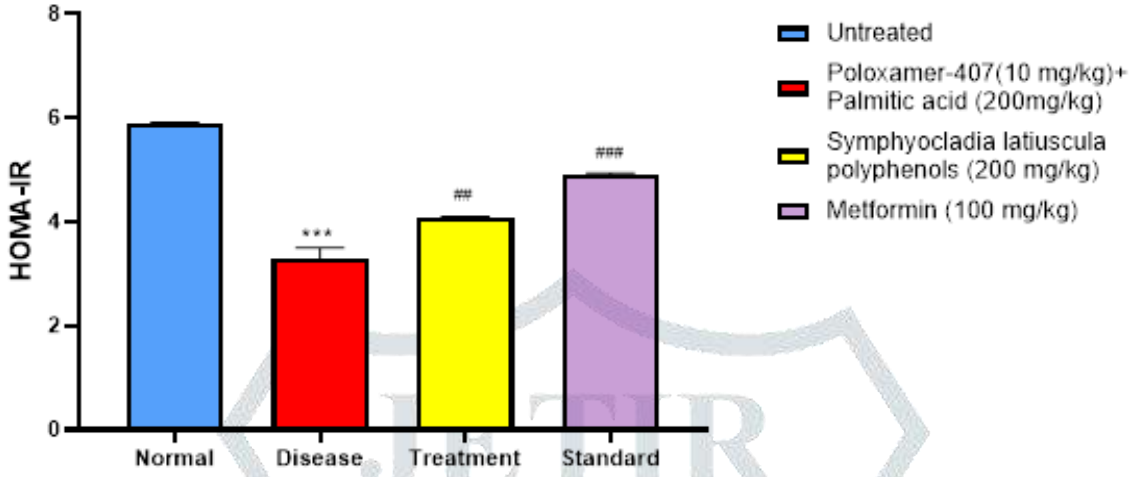


Fig 6 Homeostasis Model of Insulin Resistance (HOMA-IR)
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.01$) versus disease control
Significant ($p < 0.001$) versus disease control

Insulin Assay

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant reduction in insulin levels (6.928±0.3404 mIU/ml; $p < 0.001$) compared to normal control (untreated) (23.76±0.4093 mIU/ml). SL treated group (15.41±0.4579 mIU/ml; $p < 0.001$) and standard group (20.04±0.6533 mIU/ml; $p < 0.001$) showed significantly reduced levels compared to disease control.

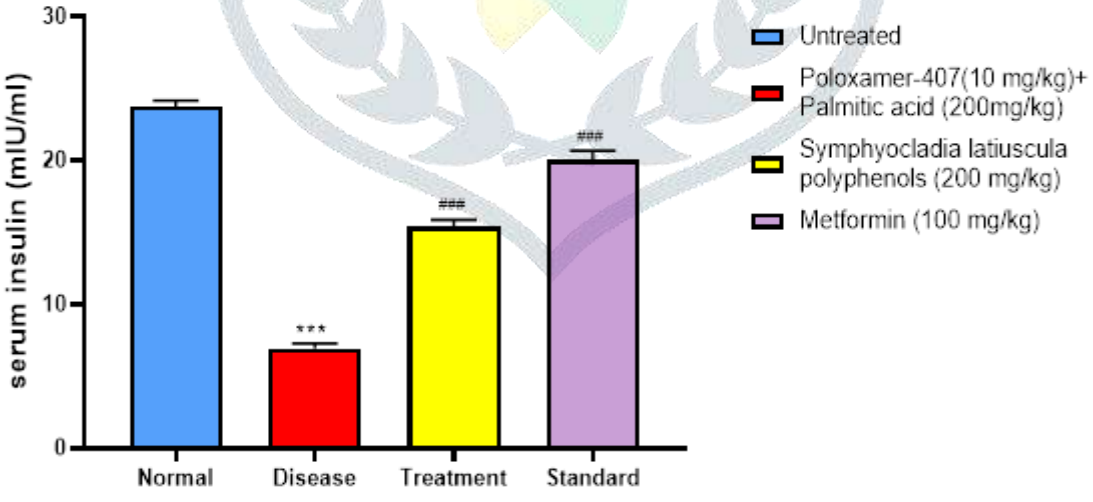


Fig 7 Insulin Assay
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

Biochemical Parameters:

Total Cholesterol

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in total cholesterol levels (129.8±1.062 mg/dL; $p < 0.001$) compared to normal control (untreated) (58.89±0.5678 mg/dL). SL treated group (71.92±1.110 mg/dL; $p < 0.001$) and standard group (64.97±0.5087 mg/dL; $p < 0.001$) showed significantly reduced levels compared to disease control.

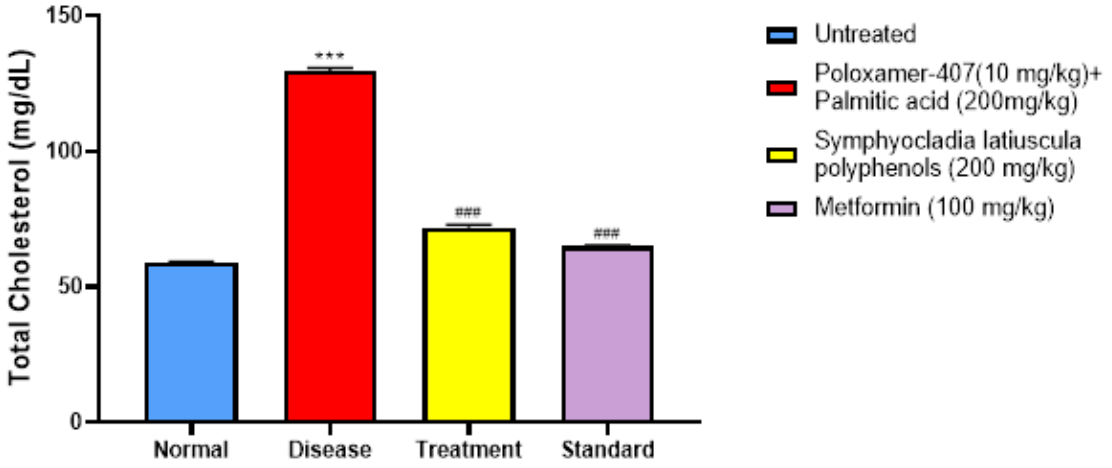


Fig 8 Total cholesterol
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

Triglycerides

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in triglycerides levels (144.3± 1.984 mg/dL; $p < 0.001$) compared to normal control (untreated) (63.36± 0.8179 mg/dL). SL treated group (81.52± 0.6540 mg/dL; $p < 0.001$) and standard group (73.07± 0.9366 mg/dL; $p < 0.001$) showed significantly reduced levels compared to disease control.

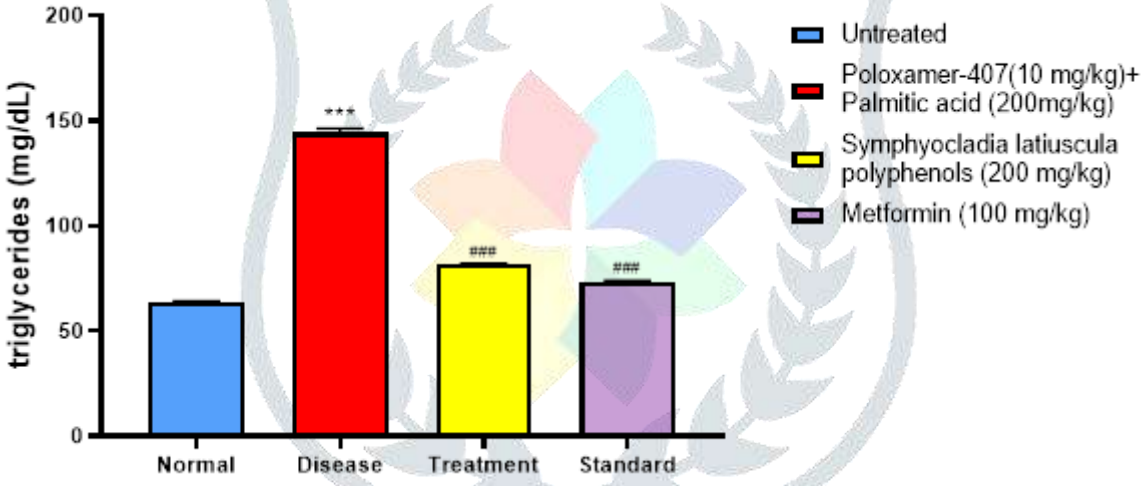


Fig 9 Triglycerides
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

HDL

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant decrease in HDL levels (26.79± 0.8769 mg/dL; $p < 0.001$) compared to normal control (untreated) (53.09±0.8622 mg/dL). SL treated group (39.27± 0.5969 mg/dL; $p < 0.001$) and standard group (44.22± 0.5799 mg/dL; $p < 0.001$) showed significantly increased levels compared to disease control.

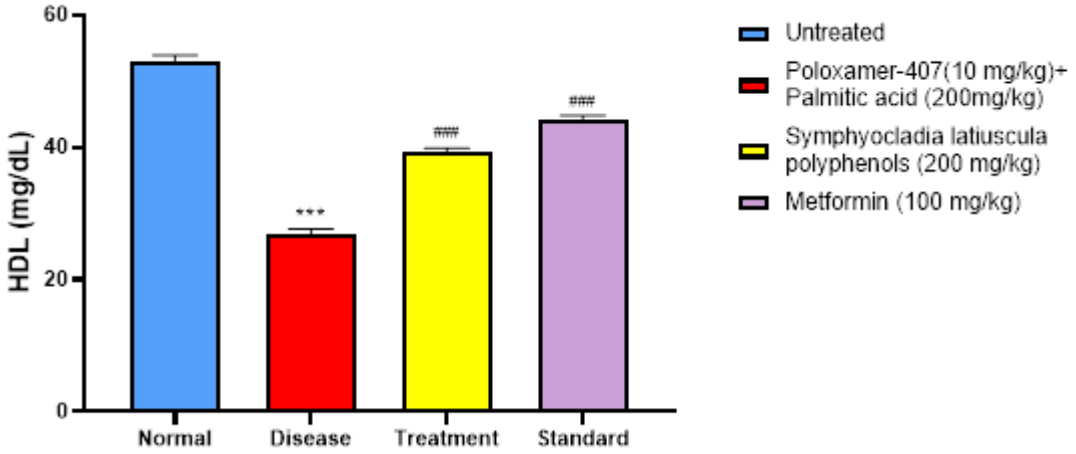


Fig 10 HDL
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

LDL

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in LDL levels (85.21±0.4804 mg/dL; $p < 0.001$) compared to untreated group (57.17±0.7622 mg/dL). SL treated group (75.19±0.9419 mg/dL; $p < 0.05$) showed dose dependent and standard group (64.47±1.231 mg/dL; $p < 0.01$) showed significant reduced levels compared to disease group.

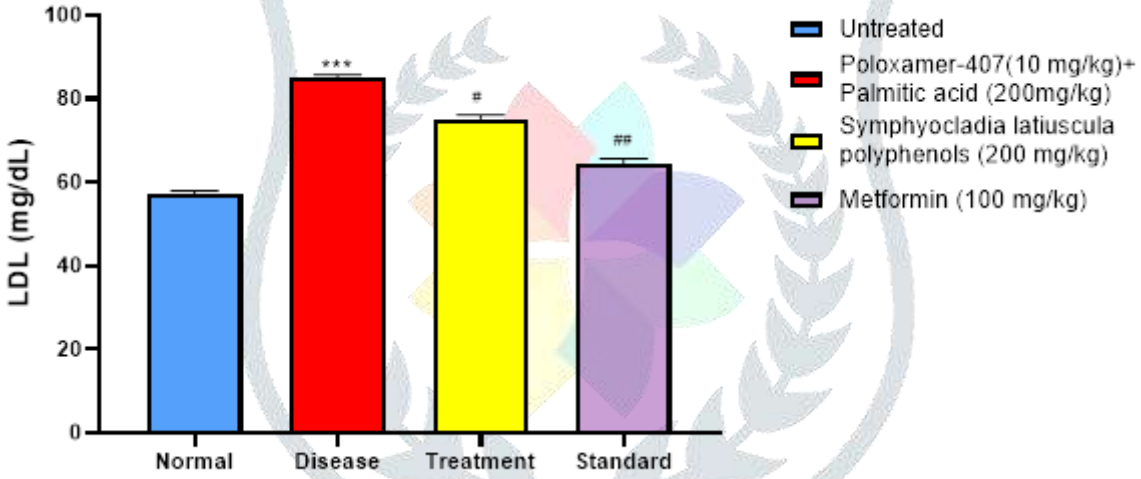


Fig 11 LDL
*** Significant ($p < 0.001$) versus normal control
# Dose dependent ($p < 0.05$) versus disease control
Significant ($p < 0.01$) versus disease control

VLDL

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in VLDL levels (39.21±0.5619 mg/dL; $p < 0.001$) compared to untreated group (19.52±0.3228 mg/dL). SL treated group (31.60±0.5980 mg/dL; $p < 0.01$) and standard group (24.69±0.7332 mg/dL' $p < 0.001$) showed significantly reduced levels compared to disease control.

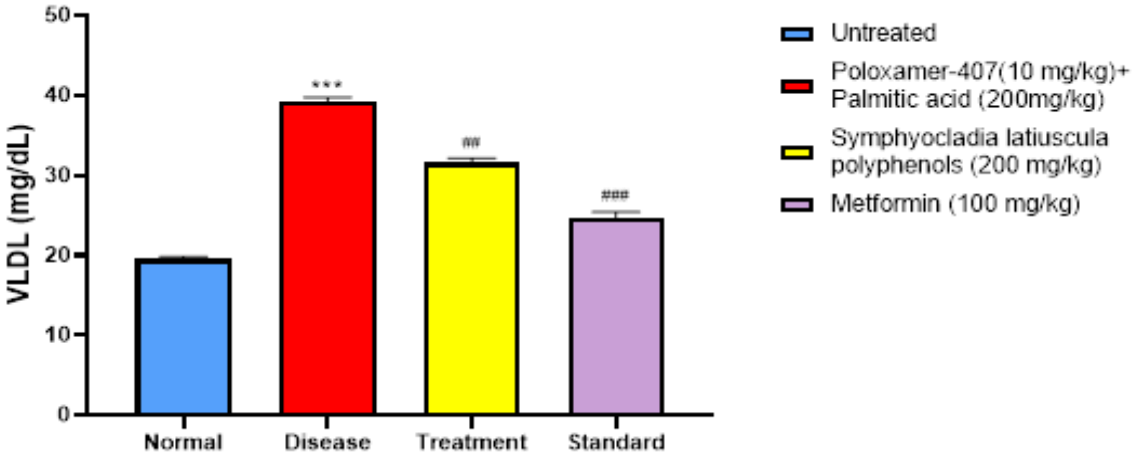


Fig 12 VLDL
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.01$) versus disease control
Significant ($p < 0.001$) versus disease control

HbA1c

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in HbA1c levels (8.753±0.1903 %; $p < 0.001$) compared to untreated group (4.633±0.1469 %). SL treated group (7.012±0.1019 %; $p < 0.01$) and standard group (5.872±0.2209 %; $p < 0.001$) showed significantly reduced levels compared to disease control.

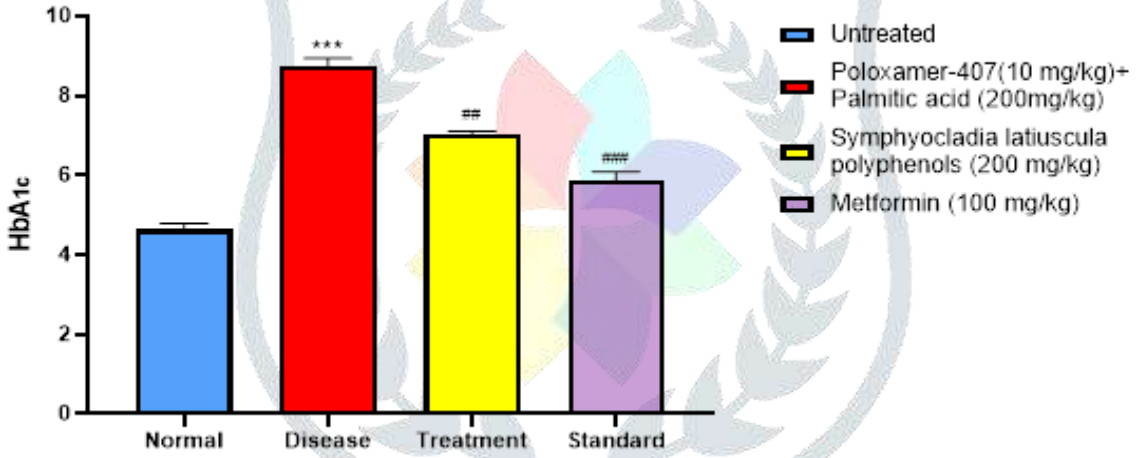


Fig 13 HbA1c
*** significant ($p < 0.001$) versus normal control
significant ($p < 0.01$) versus disease control
significant ($p < 0.001$) versus disease control

Anti-Oxidant Studies:

Glutathione

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant decrease in GSH levels (0.6228±0.16 µmol/g tissues; $p < 0.001$) compared to untreated group (3.276±0.06 µmol/g tissues). SL treated group (1.492±0.08 µmol/g tissues; $p < 0.001$) and standard group (2.399±0.11 µmol/g tissues; $p < 0.001$) showed significantly increased levels compared to disease group.

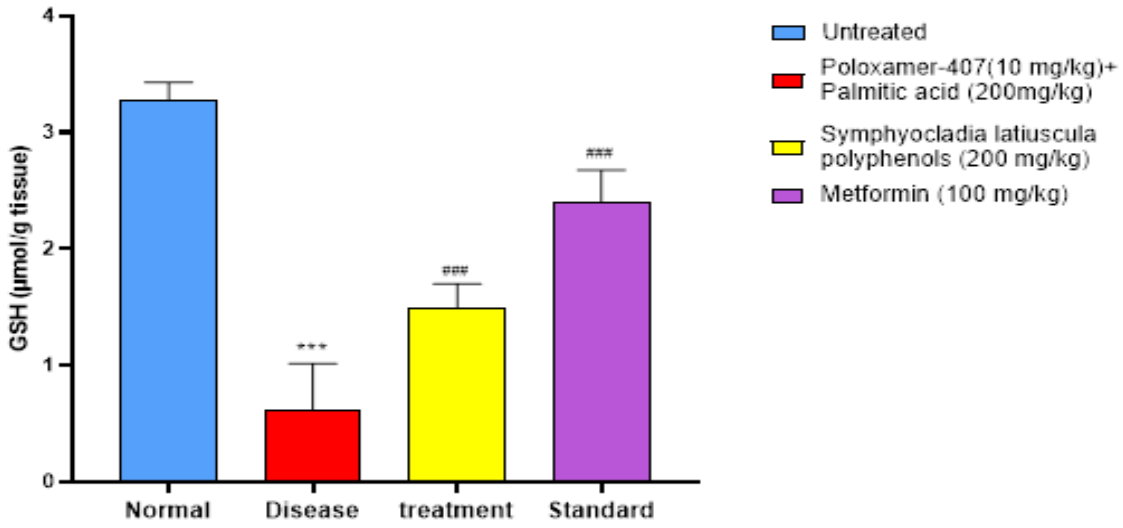


Fig 14 Glutathione
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.001$) versus disease control

Superoxide Dismutase

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in SOD levels (36.60±0.6337 U/mg; $p < 0.001$) compared to untreated group (23.98±0.4093 U/mg). SL treated group (31.02±0.3902 U/mg; $p < 0.01$) and standard group (27.02±0.3224 U/mg; $p < 0.01$) showed significantly decreased levels compared to disease control.

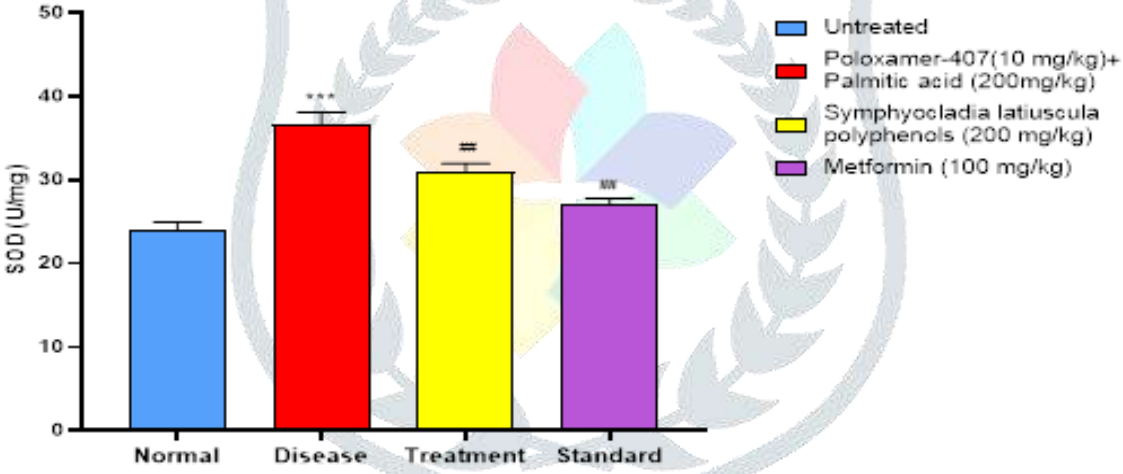


Fig 15 Superoxide dismutase
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.01$) versus disease control

Malondialdehyde

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in MDA levels (4.457± 0.1324 nmol MDA/g protein; $p < 0.001$) compared to untreated group (1.629± 0.120 nmol MDA/g protein). SL treated group (3.734± 0.1234 nmol MDA/g protein; $p < 0.01$) and standard group (2.508± 0.1570 nmol MDA/g protein; $p < 0.001$) showed significantly decreased levels compared to disease control.

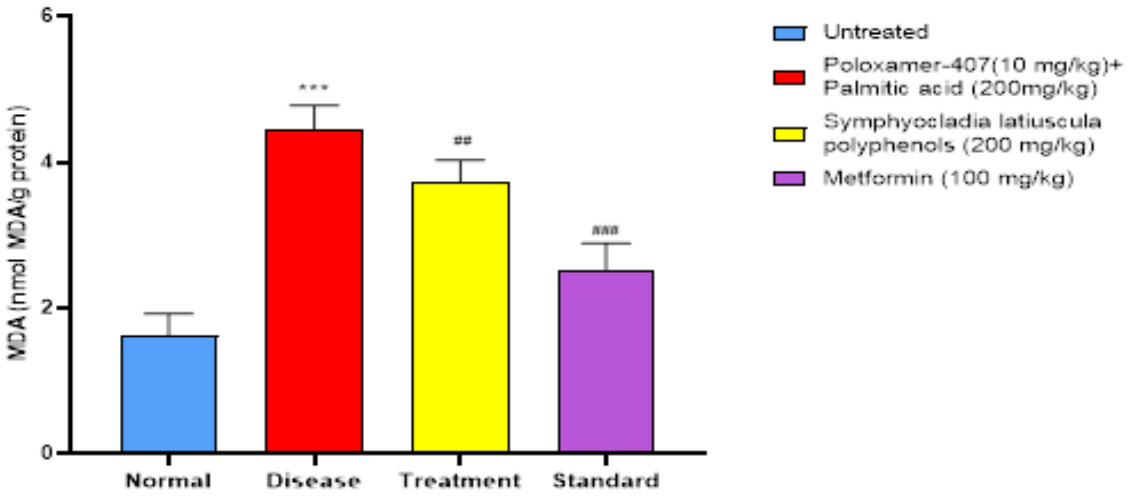


Fig 16 Malondialdehyde
*** Significant ($p < 0.001$) versus normal control
Significant ($p < 0.01$) versus disease control
Significant ($p < 0.001$) versus disease control

Nitric oxide

Induction of Palmitic Acid and Poloxamer-407 resulted in a significant increase in NO levels (4.448 ± 0.016 µmols/g protein; $p < 0.001$) compared to untreated group (1.131 ± 0.013 µmols/g protein). SL treated group (3.320 ± 0.007 µmols/g protein; $p < 0.001$) and standard group (2.229 ± 0.016 µmols/g protein; $p < 0.001$) showed significantly decreased levels compared to disease control.

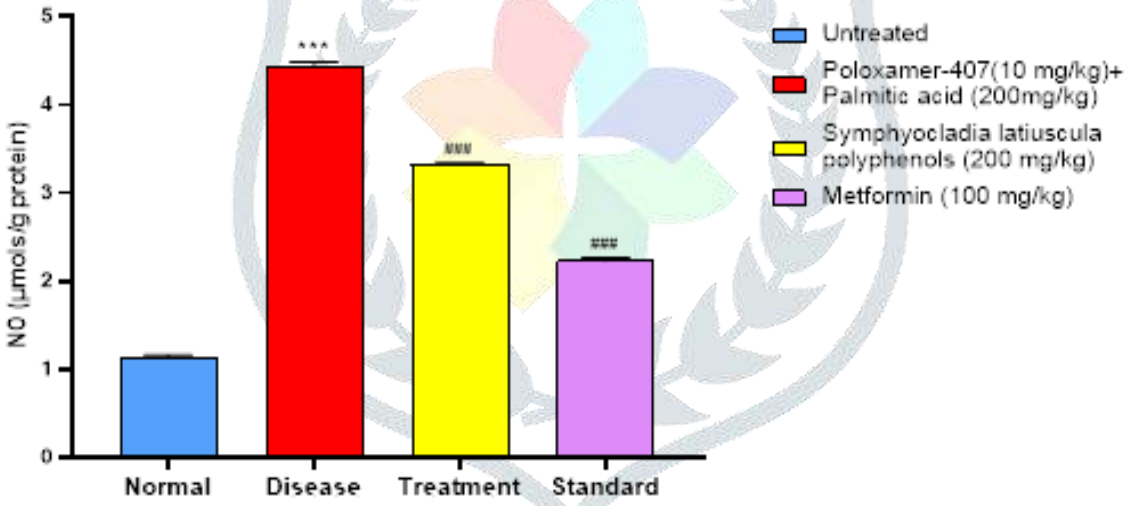
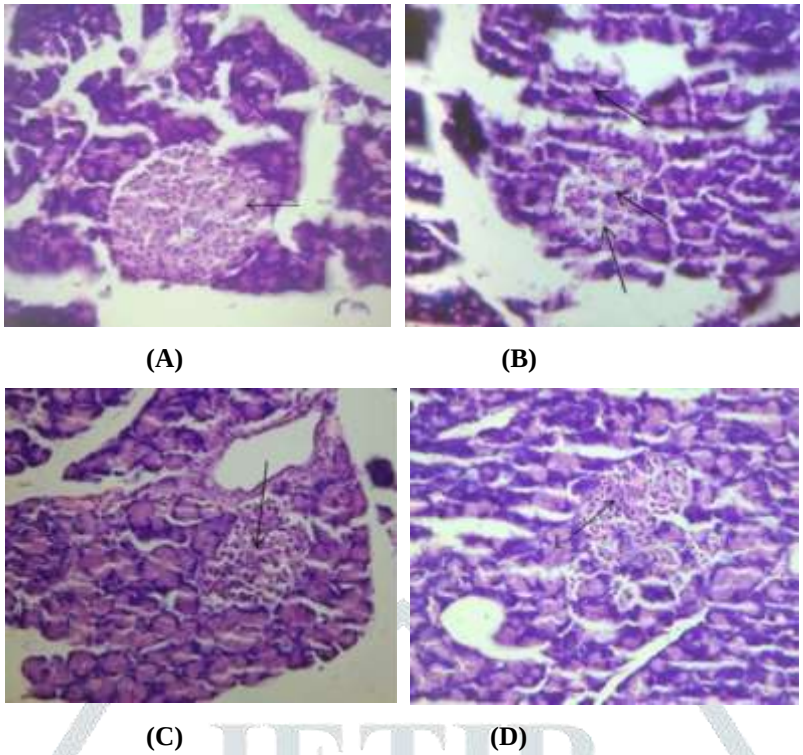


Fig 17 Nitric oxide
*** significant ($p < 0.001$) versus normal control
significant ($p < 0.001$) versus disease control

Histopathological Analysis

Histopathological examination of pancreatic sections from control group showed the normal histological structure of pancreas. The normal control group showed normal island of Langerhans with abundant basophilic cytoplasm. Presence of well demarcated pancreatic islet cells and normal acini structure in the exocrine part of the pancreas. On contrary to the normal control, the disease control group showed atrophy of the beta cells. reduction in the mass of the pancreatic islet, and overall extensive degenerative changes. Group treated with the test drug showed mild degeneration of the pancreatic islet as well as increase in the mass of the islets as compared with the disease control group. Group treated with the standard drug showed mild degeneration of the pancreatic islet as well as increase in the mass of the islets as compared with the disease control group. Pancreas showed normal sized islets of Langerhans with its normal Spale ovoid cells embedded in exocrine portion of pancreas.



(A) **(B)**

(C) **(D)**

Fig 19 Histopathological Findings
(A) Control group (B) Disease group (C) Test group (D) Standard group

DISCUSSION

Diabetes, a chronic degenerative disease, is a devastating epidemic of the 21st century and is becoming the third killer of the health of mankind after cancer, cerebrovascular and cardiovascular diseases. The side effects associated with currently available treatment options on long term basis is really an alarming concern. This study, was conducted to investigate methanolic extract of *Symphyocladia latiuscula* alga for its protective effects against Poloxamer-407 and Palmitic Acid induced pancreas dysfunction in rats.

In Fasting and Post Prandial glucose levels, induction of Poloxamer-407 and FFA (palmitic acid - 200 mg/kg, twice a day, i.p) resulted in a significant raise in fasting glucose levels in disease control compared to normal control group. SL treated group and standard group showed significantly reduced levels compared to disease control group. It also resulted in a significant raise in post prandial glucose levels compared to normal control group. SL treated group and standard group showed significantly reduced levels compared to disease control group.

Induction with Palmitic Acid and Poloxamer-407 resulted in a slight raise in sucrose preference in disease control group compared to normal control (untreated). SL treated group and standard group showed slightly reduced levels compared to disease control. However, the p value was non-significant.

In Glucose Tolerance Test, it resulted in dose dependent deterioration in glucose tolerance in disease control group compared to normal control (untreated). SL treated group and standard group showed dose dependent improvement compared to disease control.

In Insulin Tolerance test, it resulted in dose dependent deterioration in insulin tolerance in disease control group compared to normal control (untreated). SL treated group and standard group showed dose dependent improvement compared to disease control. A significant reduction in HOMA-IR index was observed compared to normal control (untreated). SL treated group and standard group showed significantly reduced levels compared to disease control. A significant reduction was observed in insulin levels compared to normal control (untreated). SL treated group and standard group showed significantly reduced levels compared to disease control.

The Biochemical parameters tested showed a significant increase in total cholesterol, triglycerides, LDL, VLDL and HbA1c levels compared to normal control (untreated). SL treated group and standard group showed reduced levels compared to disease control. A significant decrease in HDL levels was observed compared to normal control (untreated). SL treated group and standard group showed significantly increased levels compared to disease control.

Anti-oxidant parameters tested resulted in a significant decrease in GSH levels compared to normal control (untreated). SL treated group and standard group showed significantly increased levels compared to disease group. A significant increase in SOD levels was observed compared to normal control (untreated). SL treated group and standard group showed significantly decreased levels compared to disease control.

A significant increase in MDA levels was observed compared to normal control (untreated). SL treated group and standard group showed significantly decreased levels compared to disease control. A significant increase in NO levels was observed compared to normal control (untreated). SL treated group and standard group showed significantly decreased levels compared to disease control.

Histopathological examination of pancreatic sections from control group showed the normal histological structure of pancreas. The normal control group showed normal island of Langerhans with abundant basophilic cytoplasm. Presence of well demarcated pancreatic islet cells and normal acini structure in the exocrine part of the pancreas. On contrary to the normal control, the disease control group showed atrophy of the beta cells. reduction in the mass of the pancreatic islet, and overall extensive degenerative changes. Group treated with the test drug showed mild degeneration of the pancreatic islet as well as increase in the mass of the islets as compared with the disease control group. Group treated with the standard drug showed mild degeneration of the pancreatic islet as well as increase in the mass of the islets as compared with the disease control group. Pancreas showed normal sized islets of Langerhans with its normal Spale ovoid cells embedded in exocrine portion of pancreas.

CONCLUSION

The present study was carried out to investigate the protective effects of the methanolic extract of *Symphyocladia latiuscula* against Poloxamer-407 and Palmitic Acid induced pancreas dysfunction in rats. Based upon the results of the present study, we concluded that the treatment with S. *latiuscula* provided significant and comparable protective effects to metformin against Poloxamer-407 and Palmitic Acid induced pancreas dysfunction.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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