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Research Article

Evaluation of Anti-Diabetic Activity of the Combined Extract of Gurmar Leaves and Lemon Peel in Streptozocin Induced Diabetic Rats

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Abstract:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The increasing prevalence of diabetes and the adverse effects associated with long-term use of synthetic anti-diabetic drugs have encouraged the search for safer and more effective herbal alternatives. The present study was undertaken to evaluate the anti-diabetic activity of the combined extract of Gurmar leaves (Gymnema sylvestre) and Lemon peel (Citrus limon) in streptozotocin-induced diabetic rats. The plant materials were collected, authenticated, shade dried, and subjected to extraction using suitable solvents. Preliminary phytochemical screening of the combined extract revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, and terpenoids, which are known to possess significant pharmacological activities. Acute oral toxicity studies were performed according to OECD guidelines and the extract was found to be safe at the selected dose levels. Experimental diabetes was induced in Wistar rats using streptozotocin (STZ). The animals were divided into different groups including normal control, diabetic control, standard drug-treated group, and extract-treated groups receiving low and high doses of the combined extract. Treatment was continued for the specified experimental period and various biochemical parameters were evaluated, including blood glucose level, body weight, lipid profile, liver function markers, and antioxidant parameters. The results demonstrated that administration of the combined extract significantly reduced blood glucose levels in diabetic rats when compared with the diabetic control group. The extract also improved body weight, restored altered lipid parameters, and showed beneficial effects on antioxidant enzyme levels. Histopathological examination of pancreatic tissue revealed partial regeneration and protection of β-cells in extract-treated groups. The anti-diabetic effect of the combined extract may be attributed to the synergistic action of bioactive phytoconstituents present in Gymnema sylvestre and Citrus limon, which possess antioxidant and pancreatic protective properties. The study concludes that the combined extract of Gurmar leaves and Lemon peel exhibits significant anti-diabetic activity in streptozotocin-induced diabetic rats and may serve as a promising natural therapeutic agent for the management of diabetes mellitus. Further studies are required to isolate the active constituents and establish the exact mechanism of action.

Keywords: Diabetes mellitus, Gymnema sylvestre, Citrus limon, Streptozotocin, Anti-diabetic activity, Herbal extract, Wistar rats.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia arising from impaired insulin secretion, insulin action, or both. Glucose normally enters cells under the action of insulin secreted by pancreatic β-cells; when tissues become resistant to insulin or insulin secretion declines, glucose accumulates in the bloodstream, leading to metabolic dysfunction and progressive tissue damage. Uncontrolled hyperglycemia contributes to oxidative stress, advanced glycation end-product (AGE) formation, and endothelial and mitochondrial dysfunction, ultimately leading to chronic complications such as nephropathy, neuropathy, cardiovascular disease, and retinopathy.[1]

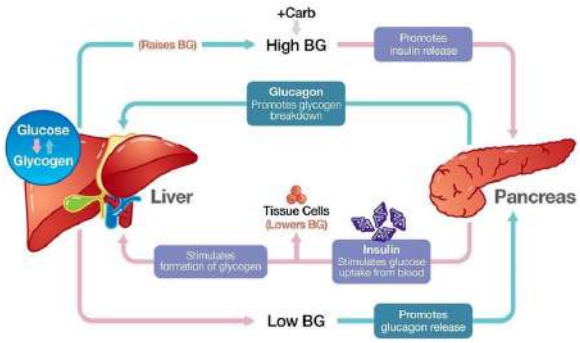


Figure 1: Diabetes Cycle

Global Prevalence of Diabetes

The prevalence of diabetes has significantly increased over the past few decades, making it a major global public health problem.

Globally, an estimated 537 million individuals (aged 20–79) had diabetes in 2021; by 2030, that figure is expected to increase to 643 million, and by 2045, it is expected to reach 783 million. The majority of occurrences of diabetes are type 2, which is frequently associated with lifestyle factors including obesity and physical inactivity Rapid urbanization, dietary transitions, and sedentary lifestyles significantly contribute to this rising prevalence.[2]

Plant Profile

Gymnema sylvestre

Figure 2: Gymnma Sylvestre Plant

Pharmacological Activities [4]

Potent antidiabetic effect:

Exhibits strong glucose-lowering activity by promoting β-cell regeneration, enhancing insulin secretion, and delaying intestinal glucose absorption.

Anti-inflammatory and antioxidant effects: Possesses significant free-radical scavenging and inflammation-reducing properties due to its rich flavonoid and phenolic content.

Anticancer and cytotoxic activity: Shows cytotoxic and antiproliferative effects through triterpenoids and gymnemic acids that induce apoptosis in cancer cells.

Antimicrobial and antifungal activity: Demonstrates broad-spectrum antibacterial and antifungal activity attributed to tannins, saponins, and flavonoids.

Antihyperlipidemic and hepatoprotective actions: Helps reduce cholesterol, triglycerides, and LDL while improving liver function and protecting hepatocytes from oxidative damage

Immunostimulatory and wound-healing activity: Enhances immune response and accelerates wound contraction, collagen synthesis, and epithelial repair, aiding diabetic wound healing.

Citrus limon

Figure 3: Citrus Limon Plant Pharmacological Activities of Citrus limon[7,8]

Antioxidant activity: Lemon peel exhibits strong antioxidant properties due to its high content of vitamin C, flavonoids, and limonoids, which help neutralize free radicals and reduce oxidative stress.

Antidiabetic effects: Extracts inhibit key carbohydrate-digesting enzymes such as α-amylase and α-glucosidase and enhance insulin sensitivity, contributing to better glycemic regulation.

Antimicrobial and antifungal activity: Lemon peel contains bioactive compounds with proven antibacterial and antifungal effects, helping inhibit microbial growth.

Anti-inflammatory and analgesic effects: Essential oil constituents like limonene and β-pinene reduce inflammation and provide mild analgesic effects.

Cardioprotective and antihyperlipidemic actions: Lemon flavonoids help lower cholesterol, improve lipid profile, and support cardiovascular health by reducing oxidative damage and improving endothelial function.

Anticancer properties: Compounds such as limonene and citral demonstrate cytotoxic and antiproliferative effects against various cancer cell lines.

Aromatherapy benefits: Lemon essential oil improves mental alertness, reduces fatigue, and promotes relaxation when inhaled.

Materials and Methods

Collection of drugs

Fresh leaves of Gymnema sylvestre were obtained from a local medicinal plant market in Jaipur, Rajasthan, India, during the month of

January. Fresh lemons (Citrus limon) were purchased from the local market in Jaipur.

Institutional animal ethics committee: Sprague Dawley male Rats served as the study's experimental animals.

The Institutional Animal Ethics Committee **(**IAEC/DPS/SU/2304), OECD guidelines, and the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Animal Welfare Division, Social Justice and Empowerment, Government of India, New Delhi, India. Were strictly followed in all procedures involving animals.

Animal grouping

The animals were divided into 6 groups of 6 rats each. They were fed a standard pellet diet and RO water. It has become possible to calculate the dose of medicine to be given to rats by converting the human dose to the animal dose.

Table 1: group of animals with selected drug doses

Groups	Tretment	No. of Animals
Group I	Normal Control	6
Group II	Disease Control	6
Group III	Disease + Gurmar Extract	6
Group IV	Disease + Lemon Peel Extract	6
Group V	Disease + Combine Gurmar and Lemon Peel Extract	6
Group VI	Diabetic+ Standard antidiabetic drug	6

Induction of Type 2 Diabetes mellitus (T2DM) - chemical-induced model

The 15 minutes before administering STZ (60 mg/kg b. w..), which was dissolved in buffer citrate (pH 4.5) just before use, nicotinamide (120 mg/kg b. w.) dissolved in saline was administered intraperitoneally. Rats with mild diabetes who had hyperglycemia and blood sugar levels below 135 mg/dl after one week were employed in the study. From the Reteo orbital plexus, blood was taken. Both vehicles received access to controls (Pierre et al., 2012)

Collection of blood sample

Blood samples had been collected from the retro-orbital plexus and placed in sterile, dry centrifuge tubes with EDTA. Plasma was extracted from blood after collection by centrifuging it at 4000 rpm for 15 minutes, and it was then kept at -20 °C until the analysis was done.

Biochemical Estimation of blood plasma

Plasma Glucose

The main carbohydrate found in blood is glucose. The body gets its energy from the process of oxidation in the cells. Diabetes mellitus, excessive parathyroid hormone, pancreatitis, and renal failure all have elevated glucose levels. Hypothyroidism, hypopituitarism, Insulinoma, and severe liver disease all have low levels.

Total protein

Proteins are components of the body's muscles, enzymes, hormones, and many other essential structural and functional components. They play a part in preserving the regular flow of water throughout the tissues and the blood. The fractions, which are mostly composed of albumin and globulin, fluctuate extensively and independently in illnesses. Increased levels are mostly associated with dehydration. Malnutrition, poor production of proteins, protein losses via haemorrhage, and excessive protein catabolism are the major causes of decreased levels.

Triglyceride (TG)

Triglyceride testing is crucial for the diagnosis and treatment of hyperlipidaemias. Heart, liver, and kidney damage are all linked to high triglyceride levels. Triglycerides are a single

reagent set that includes the enzymes lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase for determining triglycerides.

Total cholesterol (TC)

The major lipid present in blood, bile, and brain tissue is cholesterol. It is the primary lipid linked to vascular disorders caused by atherosclerosis. It is necessary for the synthesis of cellular membranes and steroid hormones. Lipoproteins carry cholesterol into the bloodstream after it has been metabolised in the liver. Increased levels are seen in hyperlipidaemia and hypercholesterolaemia. cirrhosis, hypothyroidism, uncontrolled diabetes, and nephrotic syndrome. Low levels are associated with liver disorders, malnutrition, hyperthyroidism, anaemias, and malabsorption.

Biochemical estimation of the organ's supernatant (antioxidant enzymes) Preparation of Heart and Kidney Supernatant

The heart and kidney were taken from the animals and rinsed in cooled 0.9% saline after a large dosage of anaesthetic (Thiopental sodium) was administered intraperitoneally (i.p.) Homogenate was then made in cold phosphate buffer saline (0.05 M, pH 7.4) using a homogeniser. The resultant homogenates were centrifuged at 10,000 x g for 10 minutes at 4 °C, and the pancreatic supernatant was stored at a deep freezer temperature (-20 °C) until analysis.

Superoxide dismutase (SOD)

SOD activity was determined by how well it could prevent the autoxidation of epinephrine at an alkaline pH. After 25 ml of tissue supernatant was added to 0.1 mM epinephrine in a total volume of 1 ml of carbonate buffer (pH 10.2), adrenochrome synthesis was measured at 295 nm. The SOD activity (U/mg of protein) was calculated using the standard plot.

Lipid peroxidation (LPO)

8 gm of sodium lauryl sulphate in 100 ml of distilled water. Hydrochloric acid, 2.298 ml, in 100 ml of water, is acetic acid. 1 gm of thio-barbituric acid (TBA) in 100 ml of pH 7 Tris hydrochloric acid (TCA) buffer.

Catalase (CAT)

CAT is an enzyme that combines with H2O2 to produce water and oxygen, as well as peroxidase activity when combined with a hydrogen donor. CAT guards and detoxifies internal H2O2 at the cellular level. The Claiborne (1985) approach was used to measure the concentration of CAT in tissue homogenate. Briefly, 1.95 ml of phosphate buffer (0.05 M, pH 7.0) and 1.0 ml of H2O2 (0.019 M) were combined with 50 l of tissue homogenate. At 240 nm, absorbance (A0) was measured right away. To calculate the mean, absorbance was taken every minute. Calculations of CAT activity were done in terms of nm. H2O2 Utilised/mg protein.

Reduced glutathione (GSH)

The biological antioxidant glutathione, which is found in cells, aids in defending those cells against peroxides and free radicals. Reduced glutathione (GSH) and glutathione disulfide (GSSG) are the two states of glutathione that are present. The Sedlak and Lindsay (1968) approach was used to estimate the GSH concentration in tissue homogenate. In a nutshell, 100 L of the homogenate or pure GSH were treated with 1 ml of 0.2 M Tris-EDTA buffer (pH 8.2) and 0.9 ml of 20 mM EDTA (pH 4.7). The final combination was treated with 20 L of Ellman's reagent (10 mmol/l DTNB in methanol).

After 30 minutes of incubating at room temperature, the intensity of absorption at 412 nm was measured. The quantity of tissue GSH was determined using the pure GSH standard curve and is shown as GSH g/mg wet tissue. The results were given as mean ± SEM.

Histopathology of heart and kidney

To assess the appearance of changes, the dissected pancreas was placed overnight in 10% formalin, embedded in paraffin wax, and cut into longitudinal slices of 5 m thickness. The slices were stained with hematoxylin and eosin dye for histological analysis. A pathologist who was not

familiar with the various treatments evaluated the histology results. To assess the morphological changes, the dissected pancreas was fixed overnight in 10% formalin, embedded in paraffin wax, and cut into longitudinal slices of 5 m thickness. The slices were stained with haematoxylin and eosin dye for histological analysis. A pathologist who was unaccustomed to the various treatments evaluated the histology results.

Statistical analysis

All of the results are shown as mean standard error of the mean (SEM). One-way analysis of variance was used to examine the significance of the differences in means between control and treated animals for various parameters (ANOVA). Tukey's test was used to do post-hoc comparisons.

Results

Estimation of biochemical parameters Estimation of Anthropometric parameters

No significant change in food and water intake was observed throughout the research work; slightly higher food and water intake was observed in all animals in the disease control and treatment groups as compared to the initial days.

All animals (6 animals in 1 cage, a total of 36 animals) were fed 400 g of food and 250 ml of water daily, and their daily food and water intake was measured. And every animal was weighed once a week.

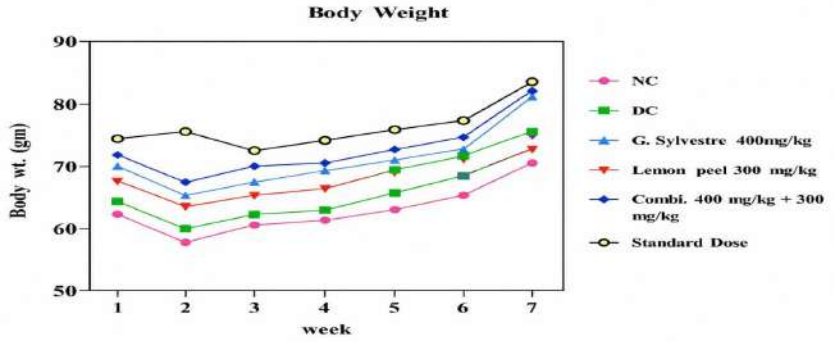


Figure 4: Food intake was s lightly increased in all group of animals

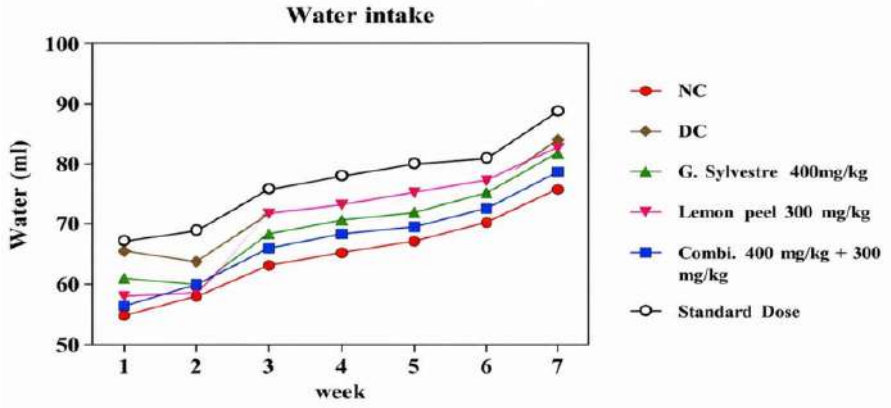


Figure 5: Water intake was slightly increased in all group of animals

Change in weight of organs: Compared to the normal control group, changes in organ weight were observed in the disease control group, and in the treatment group, compared to the disease control group, the weight of the organ decreased. It can be said that hypertrophy has occurred to some extent.

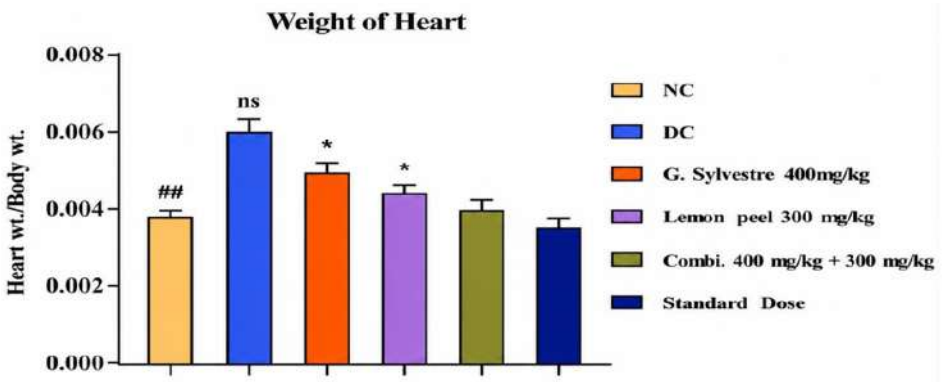


Figure 6: Organ weight - hypertrophy (Heart)

##p < 0.01 Diseases control Vs. Normal Control, ns Diseases control Vs. Gymnema Sylvestre (400 mg/kg), ns Diseases control Vs. Lemon peel (300 mg/kg), *p <0.05 Diseases control Vs. Combination dose (400mg/kg + 300 mg/kg).

End stage renal disease (ESRD)

It is commonly brought on by diabetic nephropathy (DN), a microvascular consequence of diabetes. Proteinuria, albumin secretion renal glomerular hypertrophy, enlargement of the basement membrane, malfunction of the podocytes, mesangial fibrosis, and tubulointerstitial fibrosis, among others, are the primary characteristics of DN.

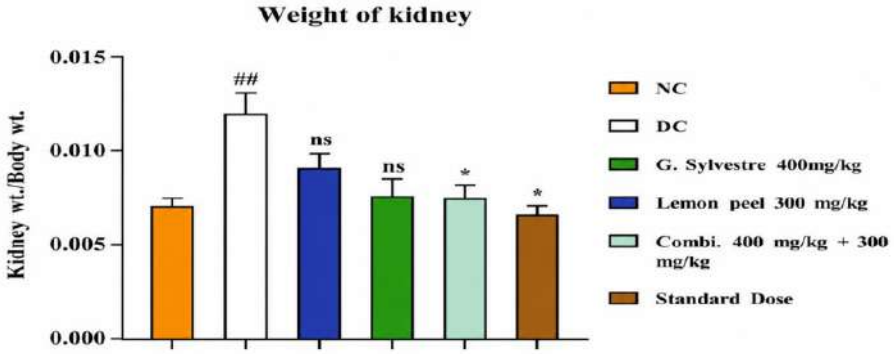


Figure 7: Organ weight - hypertrophy (Kidney)

Biochemical estimation of antioxidant parameters Superoxide dismutase (SOD) - Heart

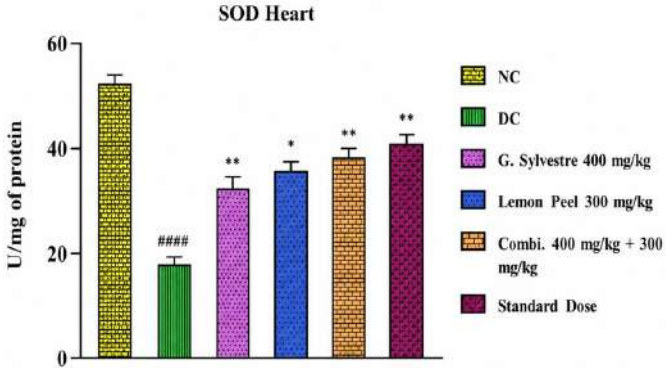


Figure 8: Effect of G.Sylvestre and Lemon Peel on SOD production in heart homogenate after 45 days treatment

####p< 0.0001 Diseases control Vs. Normal Control, **p < 0.01 Diseases control Vs. G. Sylvestre (400 mg/kg), *p < 0.05 Diseases control Vs. Lemon Peel(300 mg/kg), **p < 0.01 Diseases control Vs. Combination dose (400 mg/kg + 300mg/kg).

Superoxide dismutase (SOD) – Kidney

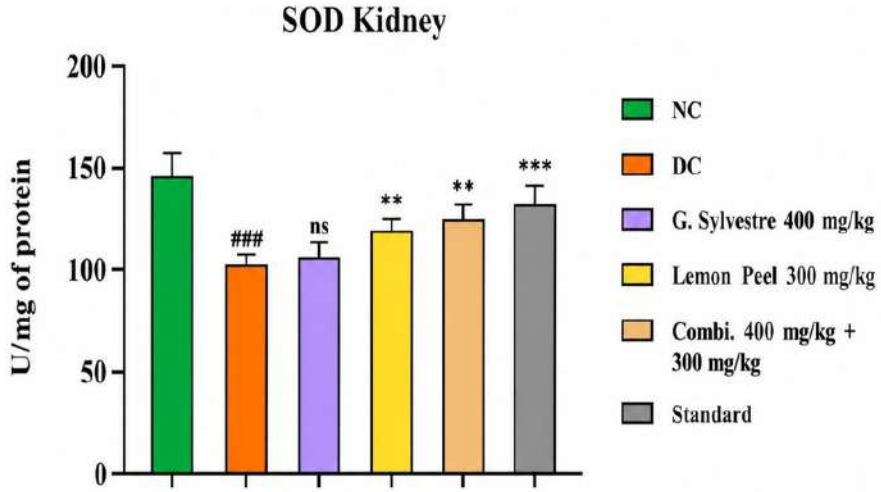


Figure 9: Effect of G.Sylvestre and Lemon Peel on SOD production in kidney homogenate after 45 days treatment

###p < 0.001 Diseases control Vs. Normal Control, ns Diseases control Vs. G.Sylvestre (400 mg/kg), **p < 0.01 Diseases control Vs. Lemon Peel(300 mg/kg),**p < 0.01 Diseases control Vs. Combination dose (400 mg/kg + 300 mg/kg),***p < 0.001 Diseases control Vs. Standard .

Catalase - Heart

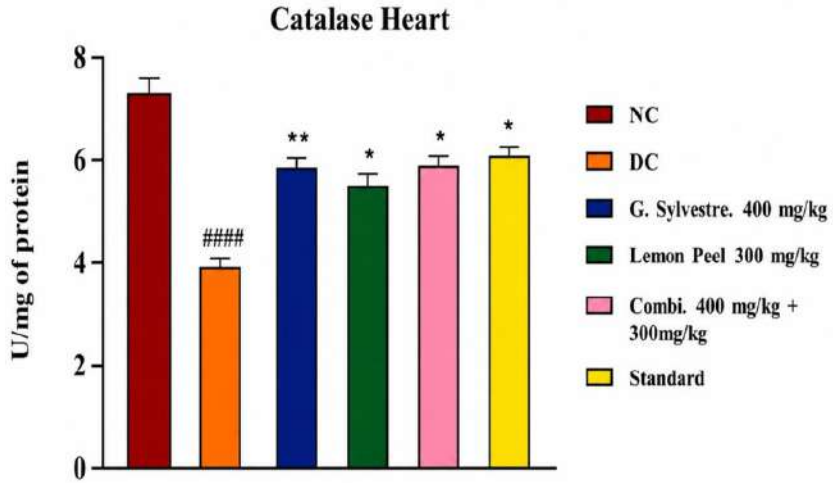


Figure 10: Effect of G.Sylvestre and Lemon Peel on catalase production in heart homogenate after 45 days treatment

####p< 0.0001 Diseases control Vs. Normal Control, **p < 0.01 Diseases control Vs. G.Sylvestre (400 mg/kg), *p < 0.05 Diseases control Vs. Lemon Peel (300 mg/kg), *p < 0.05 Diseases control Vs. Combination dose (400 mg/kg + 300 mg/kg), *p < 0.05 Diseases control Vs. Standard.

Catalase - Kidney

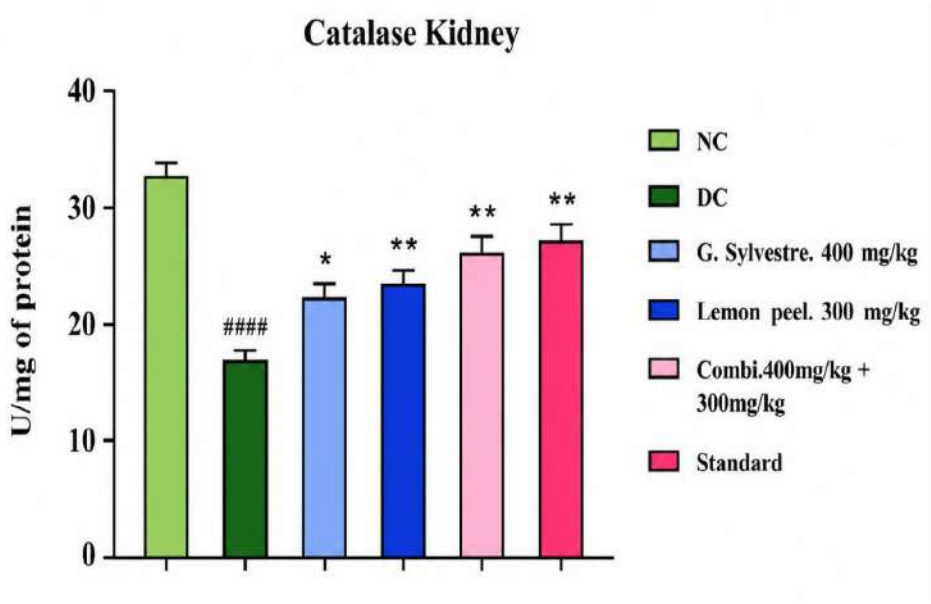


Figure 11: Effect of G.Sylvestre and Lemon Peel on catalase production in kidney homogenate after 45 days treatment

####p < 0.0001 Diseases control Vs. Normal Control, *p < 0.05 Diseases control Vs G.Sylvestre (400mg/kg), **p < 0.01 Diseases control Vs. Lemon Peel (300mg/kg), **p < 0.01 Diseases control Vs. Combination dose (400 mg/kg + 300 mg/kg), **p < 0.01 Diseases control Vs. Standard.

Glutathione (GSH) - Heart

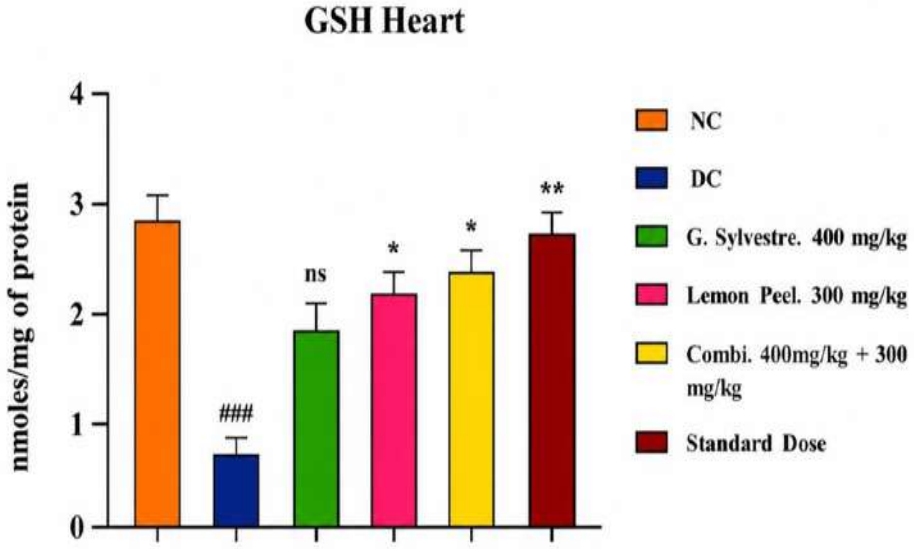


Figure 12: Effect of G.Sylvestre and Lemon Peel on GSH production in heart homogenate after 45 days treatment

###p< 0.001 Diseases control Vs. Normal Control, ns Diseases control Vs. G.Sylvestre (400 mg/kg), *p < 0.05 Diseases control Vs. Lemon Peel (300mg/kg),*p < 0.05 Diseases control Vs. Combination low dose (3 mg/kg + 5 mg/kg), **p< 0.01 Diseases control Vs. Standard.

Glutathione (GSH) - Kidney

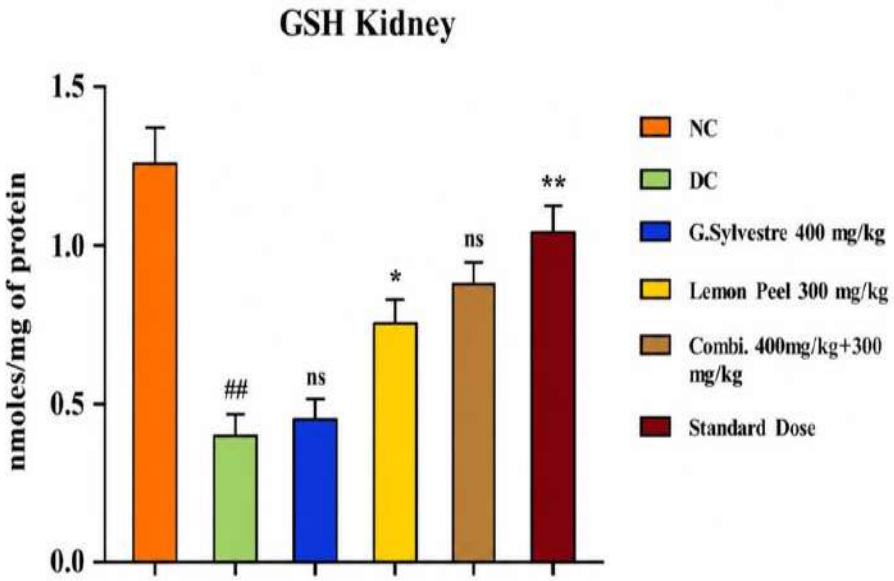


Figure 13: Effect of G.Sylvestre and Lemon Peel on GSH production in kidney homogenate after 45 days of treatment

##p < 0.01 Diseases control Vs. Normal Control, ns Diseases control Vs. G.Sylvestre (400 mg/kg), ns Diseases control Vs. Lemon Peel (300 mg/kg), *p <0.05, Disease control Vs. Combination dose (400 mg/kg + 300 mg/kg), **p <0.01 Disease control Vs. Standard dose.

Lipid peroxidation (LPO) - Heart

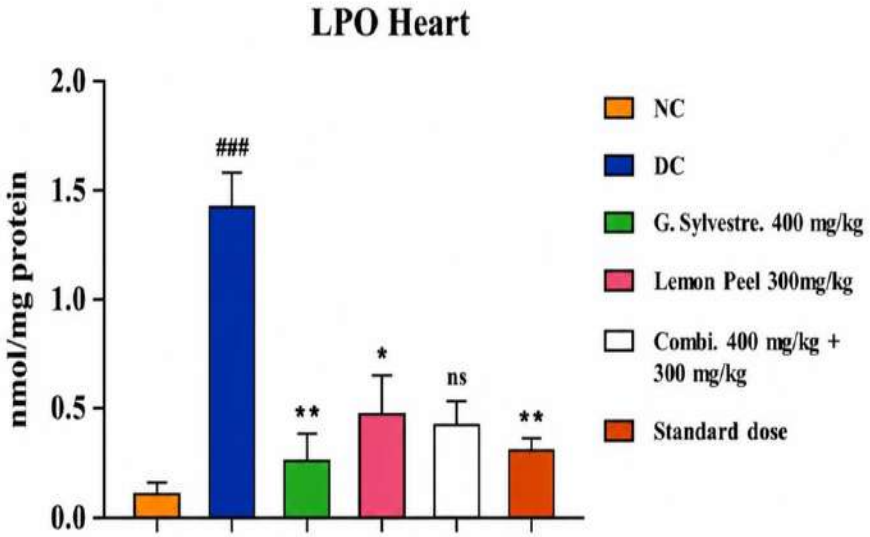


Figure 14: Effect of G.Sylvestre and Lemon Peel on LPO production in heart homogenate after 45 days treatment

###p < 0.001 Diseases control Vs. Normal Control, **p < 0.05 Diseases control Vs. G.Sylvestre (400 mg/kg), *p < 0.05 Diseases control Vs. Lemon Peel (300mg/kg), ns Diseases control Vs. Combination low dose (400mg/kg + 300 mg/kg),**p < 0.01 Diseases control Vs. Standard dose.

Lipid peroxidation (LPO) - Kidney

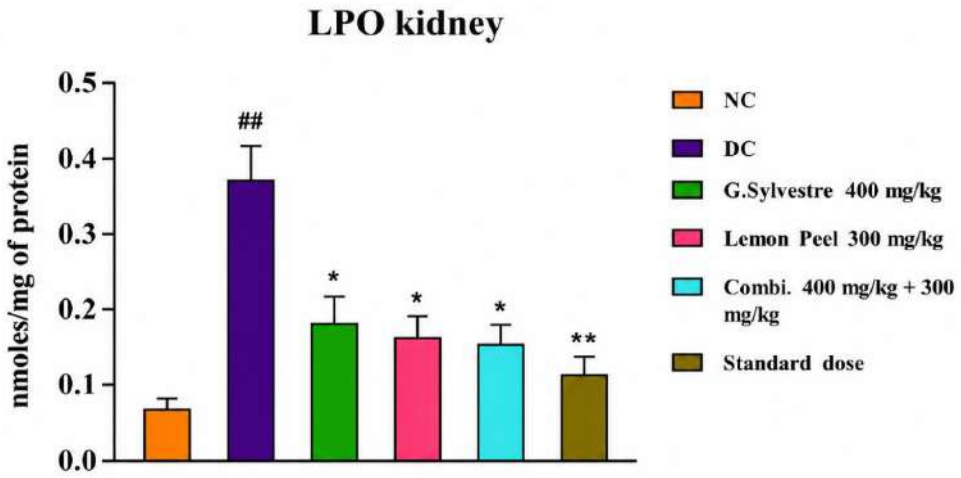


Figure 15: Effect of G.Sylvestre and Lemon Peel on LPO production in kidney homogenate after 45 days of treatment

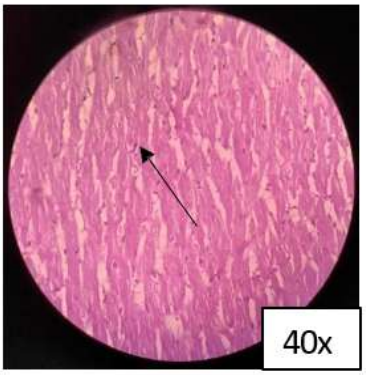
##p < 0.01 Diseases control Vs. Normal Control, *p < 0.05 Diseases control Vs. G.Sylvestre (400 mg/kg), *p < 0.05, Disease control Vs. Lemon Peel (300 mg/kg),*p < 0.01, Disease control Vs. Combination dose (400 mg/kg + 300 mg/kg), **p< 0.01, Diseases control Vs. Standard dose.

Table 1: Effect of G.Sylvestre and Lemon peel on antioxidant parameters (Heart)

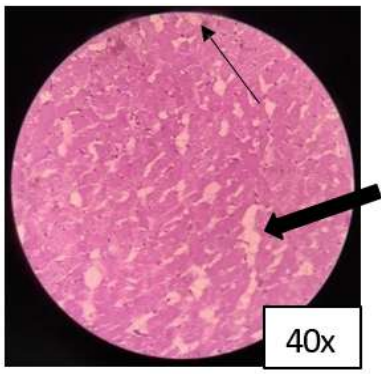
Group no.	Treatment	SOD	Catalase	GSH	LPO
I	NC	50.28 ± 0.68	7.37 ± 0.13	2.81 ± 0.15	0.15 ± 0.01
II	DC	18.90 ± 1.25$^{\#\#\#\#}$	4.04 ± 0.26$^{\#\#\#\#}$	0.74 ± 0.04$^{\#\#\#}$	1.04 ± 0.11$^{\#\#\#}$
III	Treatment I	32.46 ± 2.18**	5.76±0.11**	1.80 ± 0.27	0.22 ± 0.08**
IV	Treatment II	35.77 ± 2.03*	5.57 ± 0.14*	2.10 ± 0.30*	0.44 ± 0.20*
V	Combination dose	38.78 ± 2.64**	5.96 ± 0.19*	2.33 ± 0.28*	0.41 ± 0.19
VI	Standard Dose	42.19 ± 2.62**	6.18 ± 0.21*	2.68 ± 0.28**	0.31 ± 0.12**

Histopathological Study

Effect of G.Sylvestre and Lemon peel on Cardiac histopathological changes



(A) Control



(B) Disease Control

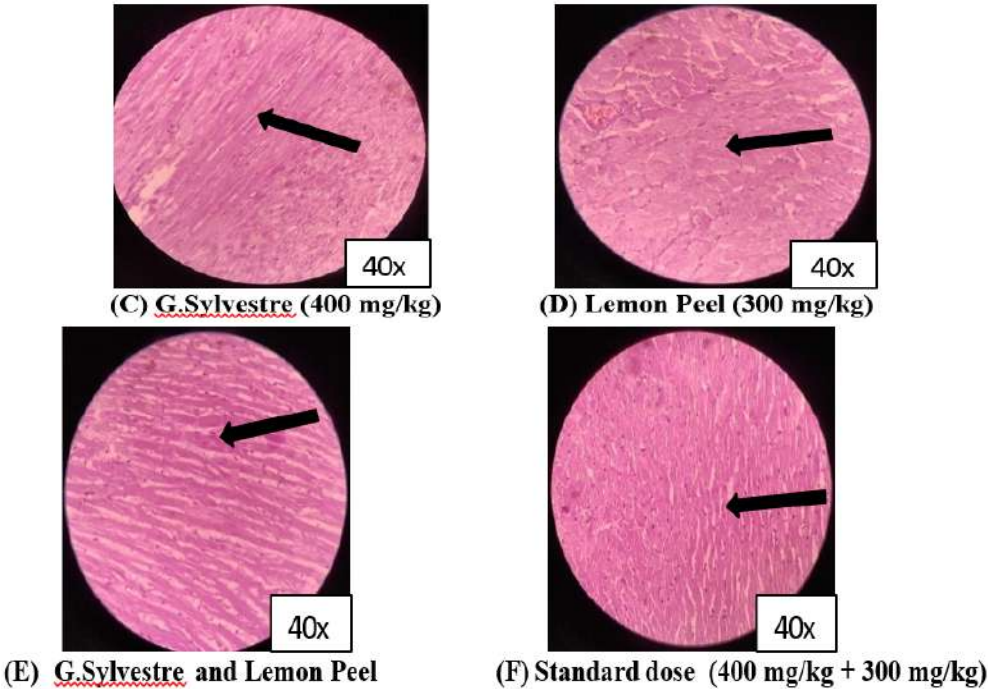
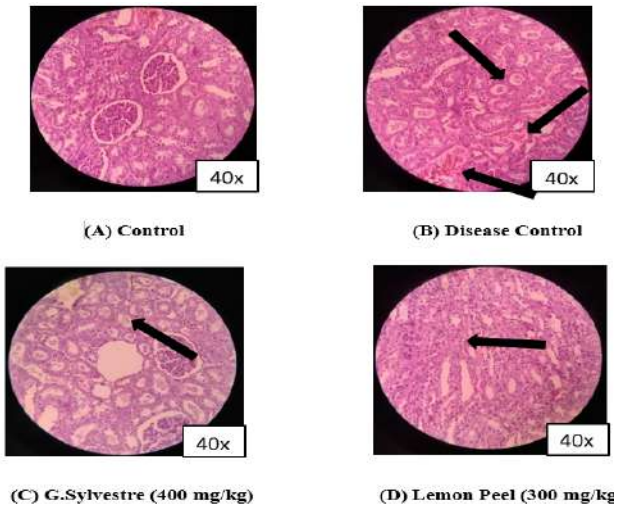


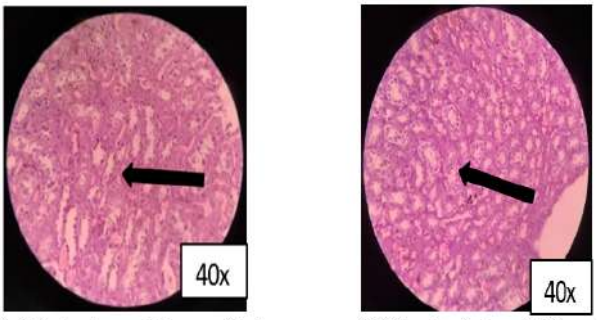
Figure 16: Histopathological features of rat's cardiac tissues

Interpretation

Shows the cardiac histopathologic characteristics of normal rat's, with empty areas showing normal myocardium space, (pink colour shows) muscle fibre, (thin aero) intercalated disk, (dark purple granules like structure) nuclei of myocytes. Shows the disease control group, with STZ induced T2DM increase myocardium space (inflammation), decrease nuclei of myocytes (necrosis) and (thin aero) tissue damage. Shows the G.Sylvestre (400mg/kg) mono therapy some improvements are seen to some extent. Like Necrosis, inflammation and tissue damage. Shows the Lemon Peel (300 mg/kg) a little recovery can be seen in it too like Necrosis, inflammation and tissue damage. Represent the G.Sylvestre and Lemon peel (400 mg/kg + 300 mg/kg) combination dose gives a good effect on diabetic heart tissue, and shows the tissue damage and absence of necrosis and inflammation.

Effect of G.Sylvestre and Lemon Peel on Renal Histopathological Changes





(E) G.Sylvestre and Lemon Peel (F) Standard dose (400 mg/kg + 300 mg/kg)

Figure 17: Histopathological features of the rat's renal tissues

Interpretation

Shows the renal histopathologic characteristics of normal rats, with (dark pink big circles) renal tubules, glomeruli, and epithelial cells. shows the disease control group, with STZ-induced T2DM, increased thickening of the capillary wall (inflammation), necrosis and (thick aero) tissue damage. Shows the G.Sylvestre (400mg/kg) mono therapy, some improvements are seen to some extent.

Decrease Necrosis, inflammation and presence of some tissue damage. Shows that the Lemon peel (300mg/kg), a recovery can be seen in it. like a little amount of Necrosis, inflammation and tissue damage. Represent the G.Sylvestre and Lemon peel (400mg/kg + 300 mg/kg) combination, low dose gives a good effect on diabetic renal tissue, and shows the tissue damage and absence of necrosis and inflammation.

Represent the Standard dose is very effective on diabetic renal tissue, and shows minor inflammation and tissue damage & absence of necrosis. The recovery of the renal tissue is close to the control group.

Conclusion

In conclusion, Gymnema sylvestre and lemon peel extracts exhibit promising antioxidant and antidiabetic potential, with observable protective effects against diabetes-induced oxidative stress in cardiac tissue. However, their combination did not demonstrate enhanced efficacy in lipid peroxidation reduction, indicating the need for further mechanistic and dose-optimization studies to fully understand their interaction and therapeutic value.

Reference

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