Article

Gymnema Sylvestre Supplementation Restores Normoglycemia and Corrects Dyslipidemia through Transcriptional Modulation of ßeta Cells and Hepatocytes in the Alloxan-Induced Hyperglycemic Rats

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Abstract: The effect of *Gymnema Sylvestre* supplementation on beta cell and hepatic activity was explored in an alloxan-induced hyperglycemic rat model. Adult rats were made hyperglycemic via a single inj. (i.p) of Alloxan (120mg/kg b.w). *Gymnema Sylvestre* was supplemented @250mg/kg and 500mg/kg b.w. Blood glucose levels were constantly monitored. After 21 days, rats were euthanized, and blood and tissues (pancreas and liver) were collected for biochemical, expression, and histological analysis. One-way ANOVA was used to compare the means of different treatment groups. *Gymnema Sylvestre* significantly reduced blood glucose levels with a subsequent increase in plasma insulin levels in a dosage-dependent manner. Total oxidant status (TOS), malondialdehyde, LDL, VLDL, ALT, AST, triglyceride, total cholesterol, total protein, C-reactive protein, and cortisol levels were reduced significantly in alloxan-treated hyperglycemic rats supplemented with *Gymnema Sylvestre* as compared to control. Significantly raised paraoxonase, arylesterase, albumin, and HDL levels were also observed in *Gymnema Sylvestre* supplemented hyperglycemic rats. Increased mRNA expression of *Ins-1, Ins-2, Gck, Pdx1, Mafa,* and *Pax6* were observed, while decreased expression of *Cat, Sod1, Nrf2,* and *NF-kB* was observed in the pancreas. Whereas increased mRNA expression of *Gck, Irs1, SREBP1c, and Foxk1* and decreased expression of *Irs2, ChREBP, Foxo1, and FoxA2* were observed in the liver. The current study indicates the potent effect of *Gymnema Sylvestre* on the transcription modulation of the insulin gene in the alloxan-induced hyperglycemic rat model. Enhanced plasma insulin levels further help to improve hyperglycemia-induced dyslipidemia through transcriptional modulation of hepatocytes.

Keywords: hyperglycemia; dyslipidemia; oxidative stress; *Gymnema Sylvestre*

1. Introduction

Diabetes is a global metabolic disorder affecting more than 180 million individuals (irrespective of gender) annually [1]. Diabetes-induced hyperglycemia results in abnormal plasma lipid concentrations with conditions like hypercholesterolemia and dyslipidemia [2]. Abnormal levels of liver enzymes are also associated with dyslipidemia observed in diabetes [3]. Hyperglycemia also induces an increase in reactive oxygen species (ROS) concentration which results in the progression of diabetes mellitus [4-6]. Superoxide dismutase (SOD) and malondialdehyde (MDA) are considered significant oxidative stress markers responsible for diabetes [7-10]. Meanwhile, metabolic homeostasis solely relies on the effective regulation of glucose and lipid metabolism by the hepatocytes in the liver. The liver primarily relies on insulin signaling to maintain metabolic homeostasis. Abnormal insulin concentration/signaling, as observed in diabetes, limits the ability of the liver to maintain metabolic homeostasis, thus resulting in hyperglycemia and dyslipidemia [11,12].

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Gymnema Sylvestre (GS), a woody climbing plant from the Azclepiadaceae family, has been used as traditional fold medicine. Its leaves contain several bioactive constituents, including gymnemagenol, gymnemic acids, gymnomosides, gurmarin, and gymnemanol [13-16]. These bioactive constituents have been attributed to impart *in-vitro* antidiabetic properties mainly through insulin release via the modulation of incretins [17,18]. In the present study, the antidiabetic and anti-hypercholesterolemic properties of GS were reexamined in the context of transcriptional modulation of critical genes involved in the regulation of insulin, insulin signaling, carbohydrate, and lipid metabolism in a hyperglycemic rat model. Additionally, the role of GS as an efficient hepato-protecting agent was also examined through its possible modulation of antioxidant response elements.

2. Experimental Design

The study design was rigorously discussed and formally approved by university biosafety and bioethical boards per guidelines. All experimental protocols, including animal handling, were approved aforetime by the Institutional Animal Care and Ethical Committee, Government College University Faisalabad. 64 male Albino Wister rats weighing 230-250g were obtained from the Experimental Animal Breeding Station, Department of Physiology, Govt. College University Faisalabad, Pakistan. Animals were acclimatized for 1 week in optimum conditions at the facility to remain to minimize stress levels. A Chow maintenance diet (CMD) and water *ad libitum* was provided to these rats. All healthy recruited rats were randomly placed in four groups, as shown in Table 1.

Table 1. Detail of different groups and their subsequent treatment.

Groups	Treatments
Group I Normal (Negative Control) n = 16	Receiving chow maintenance diet only (CMD)
Group II Diabetic (Positive control): n = 16	Receiving CMD and pretreated with Alloxan (120 mg/kg b.w)
Group III Diabetic (Treatment group 1): n = 16	Receiving CMD + Supplemented with a dose of grinded powder of *G. sylvestre* 250 mg/kg BW in diet + Pretreated with Alloxan (120 mg/kg b.w)
Group IV Diabetic (Treatment group 2): n = 16	Receiving CMD + supplemented with a dose of grinded powder of *G. sylvestre* 500 mg/kg BW in diet + Pretreated with Alloxan (120 mg/kg b.w)

Diabetes was induced by intra-peritoneal injection of Alloxan (Sigma-Aldrich Co. USA) at a dose of 120 mg/kg b.w. Blood glucose levels were measured by tail tipping method using a glucometer. The rats with blood glucose levels exceeding 200 mg/dl were considered diabetic. *Gymnema Sylvestre* (GS) was obtained from a local botanical garden and identified by the botanist from the Department of Botany, Government College University Faisalabad. After crushing, the leaves were dried and powdered mechanically by using a mortar and pestle. Both treatment groups (III and IV) were fed with CMD mixed with powdered GS for 21 days [Table 1]. On the 21st day of the treatment, the rats from all groups were decapitated. The whole blood of each rat was collected in separate 5ml vials of blood vacutainer. Serum was extracted from these vials by centrifugation at 3000 × *g* for 10 minutes and stored at -20 °C until further usage. Pancreas and liver tissue were collected, and part of the tissues was immediately stored in RNA*later*© for expression analysis, and part of the tissue was fixed in 10% formalin for pathological examination.

Blood glucose and plasma insulin assessment

The plasma glucose levels were measured by using commercially available glucose strips and a glucometer (Accu-Chek® Instant Blood glucose monitor by Roche). The competitive ELISA was employed to measure the plasma levels of insulin. A commercially available ELISA kit was used. Specifically, the RayBio® Rat Insulin ELISA kit (CODE: ELR-

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Insulin-1) was utilized with the detection range and sensitivity of 5uIU/mL-300uIU/mL and 5uIU/mL, respectively.

Total oxidant status (TOS)

The method described by Nisar *et al.* [19] was used to assess the total antioxidant status (TOS) of the serum samples. The TOS in samples was examined with H_2O_2 standards (6.25, 3.12, 1.56, 0.78, and 0.39 μmol/L). The lowest limit of detection of the assay was 0.13 μmol H_2O_2 equal · L−1, along < 3% precision and the linearity until 200 μmol H_2O_2 equal · L−1. The coefficient of variation of intra-assay was below 10%.

Paraoxonase Activity

The enzyme's activity was examined by consuming 2 mmol · L−1 paraoxonase, which acts as a substrate for the assay. The previously described method from (18) was employed to measure the enzymatic activity. The lowest limit of detection in the assay was 80–100 U · min−1 · L−1. The initial rate and/or sensitivity of the hydrolysis were stable for up to 5 minutes, while the coefficient of variance for intra-assay was < 10%.

Arylesterase Activity

Per minute activity of arylesterase was measured by a method which is previously described (18). The lowest limit of detection in this assay was 40–55 kU · min−1 · L−1. The coefficient of variance in intra-assay was < 7%. The hydrolysis rate was steady up to 5 minutes after beginning hydrolysis.

Serum Lipid profile

Triglycerides (TG), Total Cholesterol (TC), and High-Density Lipoprotein-Cholesterol (HDL-Chol) levels were measured by commercially available kits from Crescent Diagnostic Systems, USA (Cat#: CS611-4, Cat#: CS603-10, Cat#: CS607-2 respectively). The lowest limit of detection for TG and TC assays were 2mg/dL and 3mg/dL, respectively. However, LDL- Cholesterol (LDL-Chol) and Very low-Density Lipoprotein- Cholesterol (VLDL-Chol) were measured by specific calculations (Friedwald equation: Total cholesterol − HDL cholesterol = LDL cholesterol).

Serum liver enzymes:

To measure the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum, a commercially available liquiform method kit supplied by Randox Laboratories Ltd. (BT29 4QY; Crumlin, County Antrim, UK) was used. The detection range of the AST kit was 7.20–1039 U/L. For ALT, the measuring range was 9.7–603 U/L. Linearity was maintained when the absorbance of the sample was < 0.5, and the CV was < 10%. Total protein and Albumin were also measured by using colorimetric assay through a commercially available kit by Sigma Diagnostics (Cat#: TP0100, Cat#: MAK125 respectively). The sensitivity of the kit for total protein was 16.5ug/mL with a maximum detection limit was 1000ug/mL. Intra and inter-assay CV was 2.2% and 4.5% respectively.

Real-time qPCR analysis

qPCR method was used to detect the expression of related mRNA levels in the pancreatic and liver tissue. Total RNA was extracted using the TRIzol reagent (Invitrogen, USA) and evaluated for concentration and purity through Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The total isolated mRNA was reverse-transcribed to cDNA using the RevertAid cDNA synthesis kit (Thermo Fisher Scientific, USA) according to the manufacturer's manual. Real-time qPCR (RT-qPCR) was carried out on the iQ5 Bio-RAD machine using Maxima SYBR Green/ROX qRT-PCR Master Mix (Thermo Fisher Scientific, USA). Expression profiles of *Ins-1, Pdx1, MafA, Pax6, Cat, Sod1, Nrf2, and NF-kB* were observed in the pancreas. Whereas for the liver, the expression profile of the following genes was observed: *GCK, Irs1, Irs2, Sod1, Sod2, Cat, ChREBP,*

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SREBP1c, Foxo1, FoxA2, and FoxK1. β-actin gene was used as a housekeeping/reference gene. The PCR was done for 15 seconds, annealing for 25 seconds at 52 °C, and the extension time was 20 seconds at 72°C. Specific primer sequences were used to amplify genes (Supplementary data: Table. 1).

Histological Analysis

Pancreas and liver samples were subjected to H&E staining for histological examination to microscopically examine the islets of Langerhans in the pancreas and the liver parenchyma.

Statistical Analysis

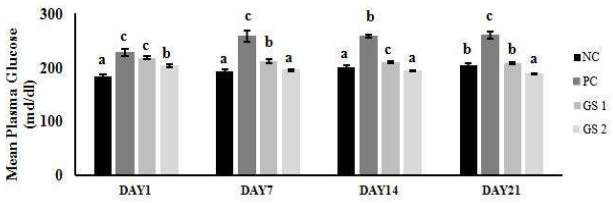
All results were expressed as mean ±SEM. One-way ANOVA was used to analyze data using SPSS statistical tool package, and Tukey's test was applied post hoc for comparing means among different groups. P≤0.05 was considered statistically significant.

3. Results

3.1. GS reduces glucose concentration and increases plasma insulin levels in a dosage-dependent manner

The glucose levels significantly declined over time in the treatment groups as compared to the positive control (Fig. 1). The dose-dependent effect was also exhibited in the treatment groups during the treatment. The glucose levels in the positive control group displayed a significant rise after 6 days as compared to the negative control group.

A



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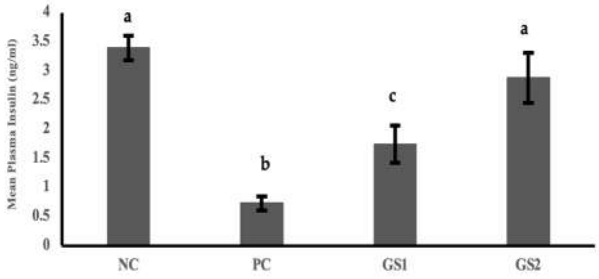

Figure 1. A) Overall ± SE mean values of serum Glucose levels in different groups. **B)** Overall ± SE mean values of serum insulin levels in different groups.
NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre,* 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant (p≥0.05).

3.2. GS reduces oxidative stress in a dosage-dependent manner

The TOS levels were significantly high in the positive control group in comparison with the negative control group (Fig. 2A). A significant dose-dependent decline was observed in TOS values in both treatment groups. The paraoxonase levels exhibited a

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significant decline in the positive control group as compared to the negative control group (Fig. 2B). *Gymnema Sylvestre* treatment groups exhibited a dose-dependent significant increase in the paraoxonase activity comparable to the negative control group. Interestingly the role of GS in mediating Superoxide dismutase (SOD) levels was not statistically significant as compared to the negative control group.

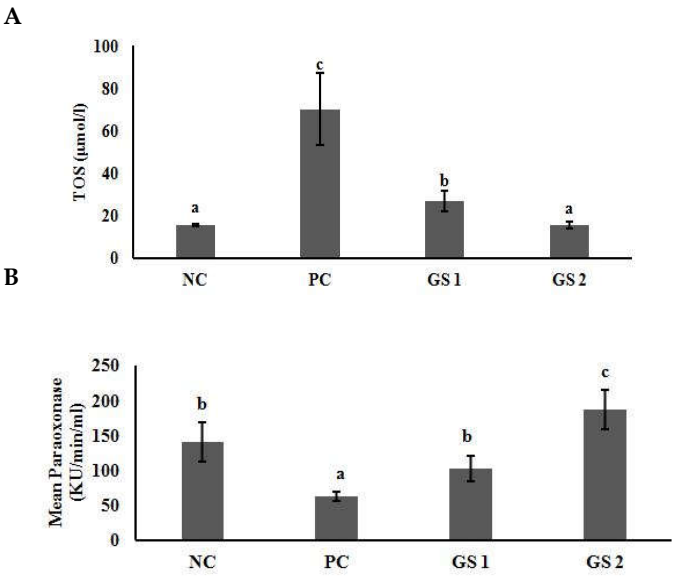

Figure 2. A) Overall ± SE mean values of Total Oxidant Status (TOS) in different groups. B) Overall ± SE mean value of Paraoxonase activity in different groups.

NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre*, 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant ($p \geq 0.05$)

3.3. GS improves hepatic response

Arylesterase activity was significantly reduced in the positive control group compared to the negative control group (Fig. 3A). *Gymnema Sylvestre* treatment significantly enhanced the arylesterase levels in a dose-dependent manner. Malondialdehyde (MDA) levels increased in the positive control group compared to the negative control group (Fig. 3B). The difference was statistically non-significant. *Gymnema Sylvestre* treatment lowered the malondialdehyde levels in a dose-dependent manner.

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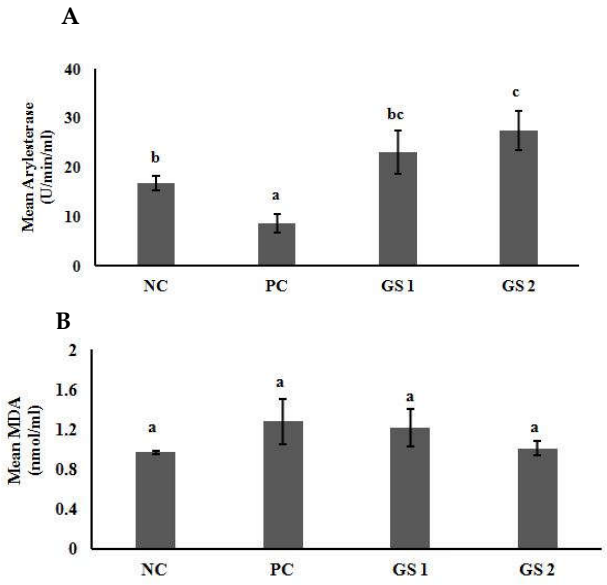

Figure 3. A) Overall ± SE mean value of Arylesterase in different groups. **B)** Overall ± SE mean value of Melanodialdehyde (MDA) activity in different groups.

NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre,* 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant (p≥0.05).

Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALT) levels were markedly increased in the positive group as compared to the negative control group (Fig. 4 A, B). A dose-dependent decline was observed only for AST in the treatment groups as compared to the positive group. The higher dose of *Gymnema Sylvestre* was found to lower the AST levels which were comparable with the negative control group levels.

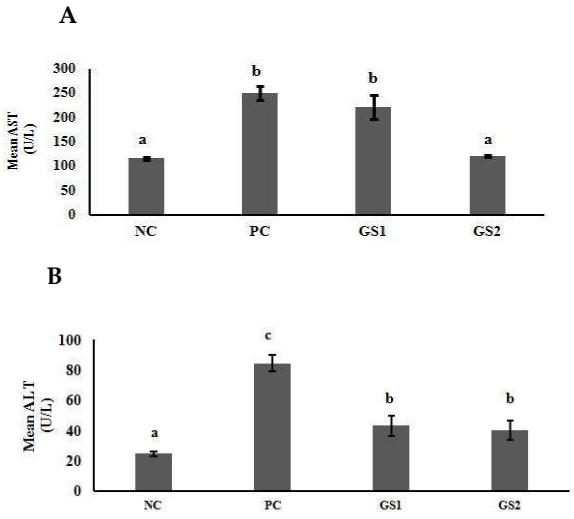

Figure 4. A) Overall ± SE mean values of Aspartate Aminotransferase (AST) in different groups. **B)** Overall ± SE mean values of Alanine Aminotransferase (ALT) in different groups.

NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre,* 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant (p≥0.05).

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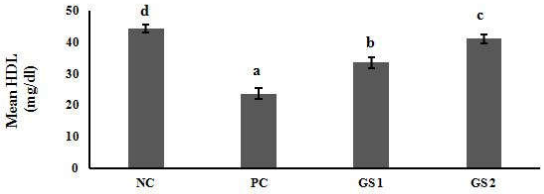
3.4. GS treatment enhanced lipid profile

High-density lipoprotein (HDL) and serum albumin levels were found to be reduced profoundly in the positive control group as compared to the negative control group (Fig. 5, A, B). Significant ($p \leq 0.05$) and dose-dependent rise was observed for HDL and albumin levels in the treatment groups as compared to the positive control group. Low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), Triglycerides, Total cholesterol and total protein levels were higher in the positive control group as compared to the negative control group (Fig. 11-15). The treatment groups exhibited a significant ($p \leq 0.05$) decline in the aforementioned parameters as compared to the positive control group. The decline observed in the treatment groups was also found to be treatment dose-dependent.

A



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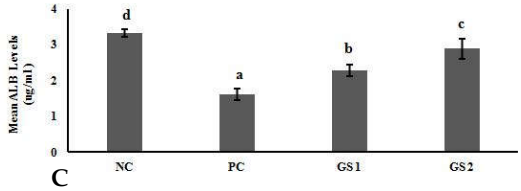

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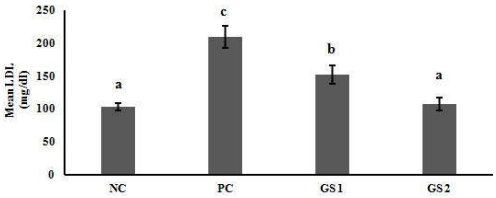

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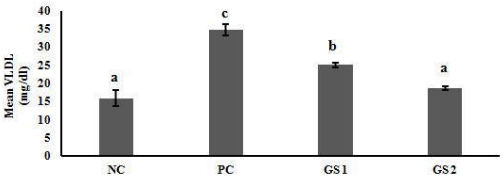

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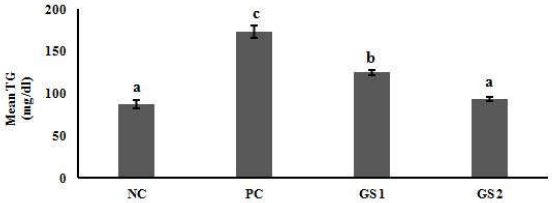

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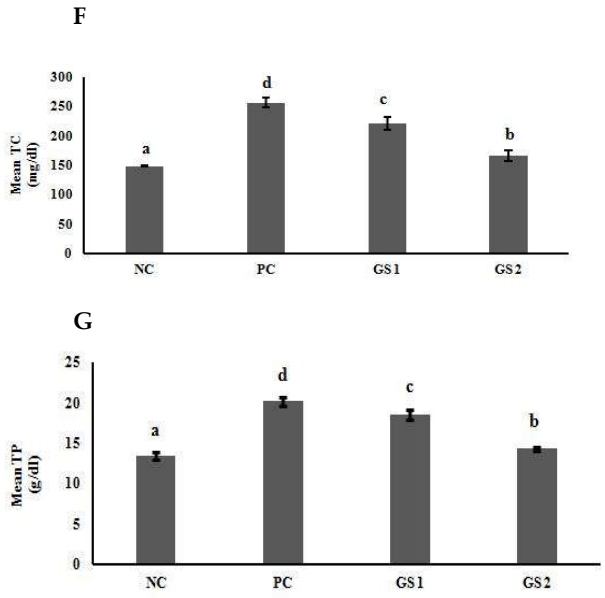

Figure 5. A) Overall ± SE mean value of High-Density Lipoprotein (HDL-CHOL) in different groups. **B)** Overall ± SE mean values of Albumin in different groups. **C)** Overall ± SE mean values of Low-Density Lipoprotein (LDL-CHOL) in different treatment groups. **D)** Overall ± SE mean levels of Very Low-Density Lipoprotein (VLDL) in different groups. **E)** Overall SE± mean values of Triglycerides (TG) in different groups. **F)** Overall SE± mean values of Total Cholesterol (TC) in different groups. **G)** Overall ± SE mean values of Total protein in (TP) different groups. NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre,* 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant (p≥0.05).

3.5. GS treatment significantly altered expression patterns in the pancreas and liver

The expression profile for the specific genes in the pancreas and liver show significant variation among different treatment groups. In the pancreas, beta cell-specific genes like *Ins1* and *Ins2* exhibit a significant decline in the positive control (PC) group followed by a gradual dose dependant significant increase in the treatment groups (GS1, GS2) (Fig. 6. A). A similar pattern of mRNA expression was observed for the glucokinase gene (*Gck*) and transcription factor genes, including pancreatic and duodenal homeobox 1 (*Pdx1),* MAF BZIP transcription factor A *(MafA),* and paired box protein 6 (*Pax6*) (Fig. 6. B). Genes responsible for the antioxidant response exhibit the same pattern of expression, where the positive control (PC) group depicts a significant decrease in the mRNA expression of catalase (*Cat*), superoxide dismutase 2 (*Sod2*/*MnSOD*), nuclear factor erythroid 2-related factor 2 (*Nrf2*). This significant increase was followed by a gradual decline in the treatment groups GS1 and GS2, however the nuclear factor kappa B (*Nf-kB)* depicts higher mRNA expression in the positive control group followed by a significant decrease in the treatmnet groups (GS1, GS2) (Fig. 6. C).

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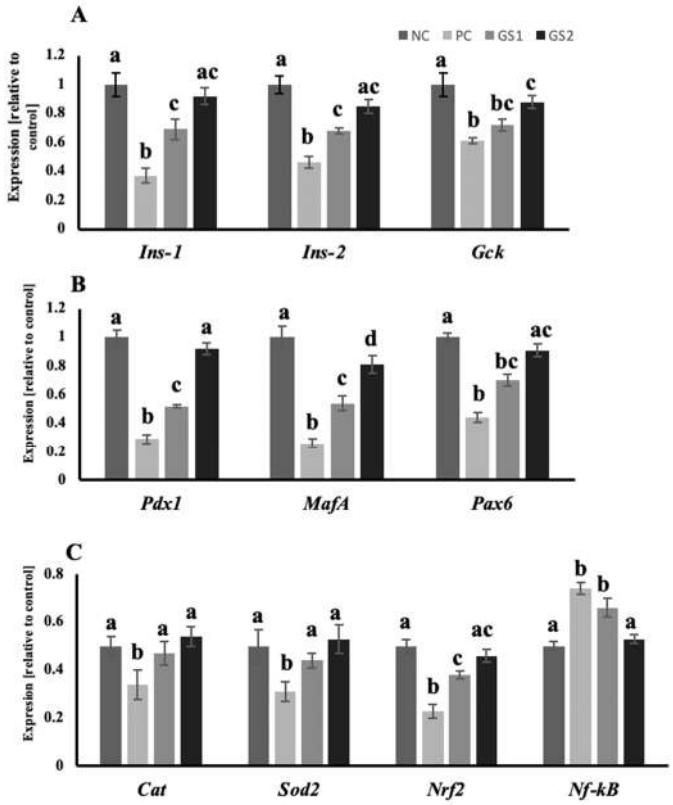

Figure 6. A) Overall ± SE mean values of *Ins1, Ins2*, and *Gck* mRNA expression in the pancreas of different groups. **B)** Overall ± SE mean values of *Pdx1, MafA,* and *Pax6* mRNA expression in the pancreas of different groups. **C)** Overall ± SE mean values of *Cat, Sod2, Nrf2,* and *Nf-kB* in the pancreas of different groups.

NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre,* 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant (p≥0.05).

In the liver tissue, the genes related to the insulin signaling in the hepatocytes, like insulin receptor substrates 1 and 2 (*Irs1, Irs2*), and the glucokinase gene (*GcK*), exhibit a distinct pattern of expression. *Irs1* and *Gck* mRNA expression declined significantly in the PC group, whereas the *Irs2* mRNA expression displayed a significant increase, followed by a gradual and significant increase in the case of *Irs1* and *Gck* and a decrease in the case of *Irs2* mRNA expression in the treatment groups GS1 and GS2 (Fig 7. A). Superoxide dismutase enzyme genes (*Sod1, Sod2*), along with the catalase (*Cat*) gene, exhibit different pattern of mRNA expression where the PC group displays a significant decrease followed by a gradual increase in both treatment groups GS1 and GS2 (Fig. 7. B). The mRNA expression pattern of Carbohydrate response element binding protein (ChREBP), a glucose-sensitive transcription factor, and sterol regulatory element binding protein 1c (*SREBP1c*), an insulin-sensitive transcription factor, displayed opposing expression patterns. The PC group exhibits a significant increase in the mRNA expression of *ChREBP* and a significant decline in the mRNA expression of *SREBP1c*. Followed by a gradual dose-dependent decline in the case of ChREBP and a dose-dependent rise in the case of *SREBP1c* mRNA expression in both treatment groups (Fig. 7. C). The mRNA expression of the insulin-sensitive Forkhead family of transcription factors, including FoxA2, Foxo1, and Foxk1, displays a distinct pattern. *FoxA2* and *FoxO1* displayed a similar expression pattern, and the

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PC group exhibited a significant increase followed by a gradual and significant decline in both treatment groups (Fig. 7. D). *Foxk1* mRNA expression displayed a significant decline in the PC group, followed by a gradual increase in the treatment groups (Fig. 7. D).

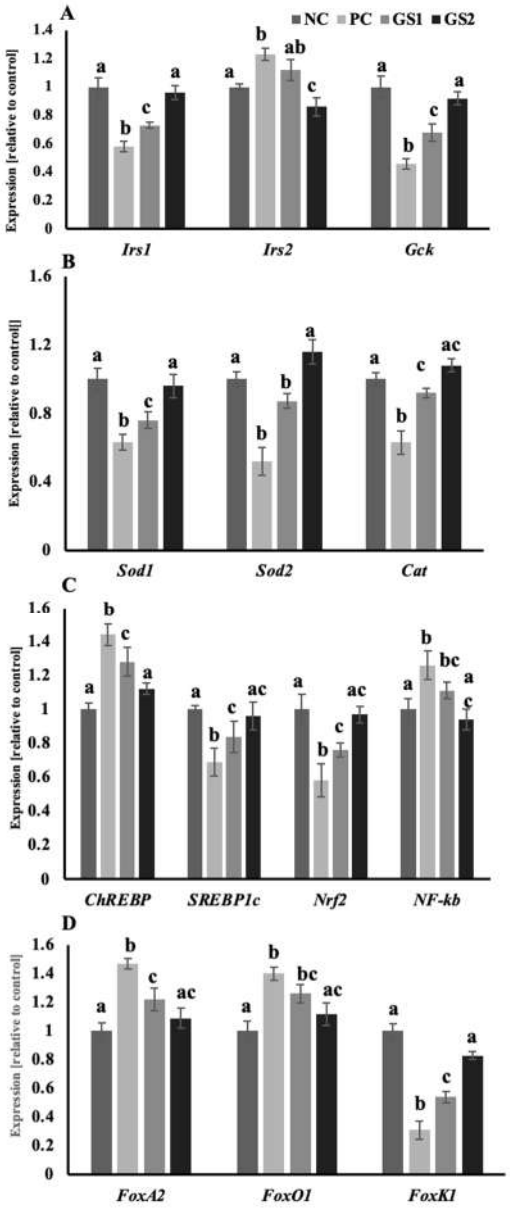

Figure 7. A) Overall ± SE mean values of *Irs1*, *Irs2*, and *Gck* mRNA expression in the liver of different groups. **B)** Overall ± SE mean values of *Sod1*, *Sod2*, and *Cat* mRNA expression in the pancreas of different groups. **C)** Overall ± SE mean values of *ChREBP*, *SREBP1c*, *Nrf2*, and *Nf-kB* mRNA expression in the liver of different groups. **D)** Overall ± SE mean values of *FoxA2*, *FoxO1*, and *FoxK1* mRNA expression in the liver of different groups.

NC = Negative control, PC = Positive control, GS1 = *Gymnema sylvestre* 250mg, GS2 = *Gymnema sylvestre*, 500mg, CMD (Chow maintenance diet). Means with similar letters are statistically non-significant ($p \geq 0.05$).

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3.6. GS treatment improves pancreatic and liver tissue histology

The microscopic analysis of the pancreatic tissue reveals a deccrease in the islet diameter and the total cell numbers in the positive control group (PC) compared to the negative control group (NC) (Fig. 8 A, B). The islets exhibit increase in their diameter and total cell numbers in the treatment groups (GS1, GS2) as compared to the positive control group (PC) (Fig. 8 C, D)

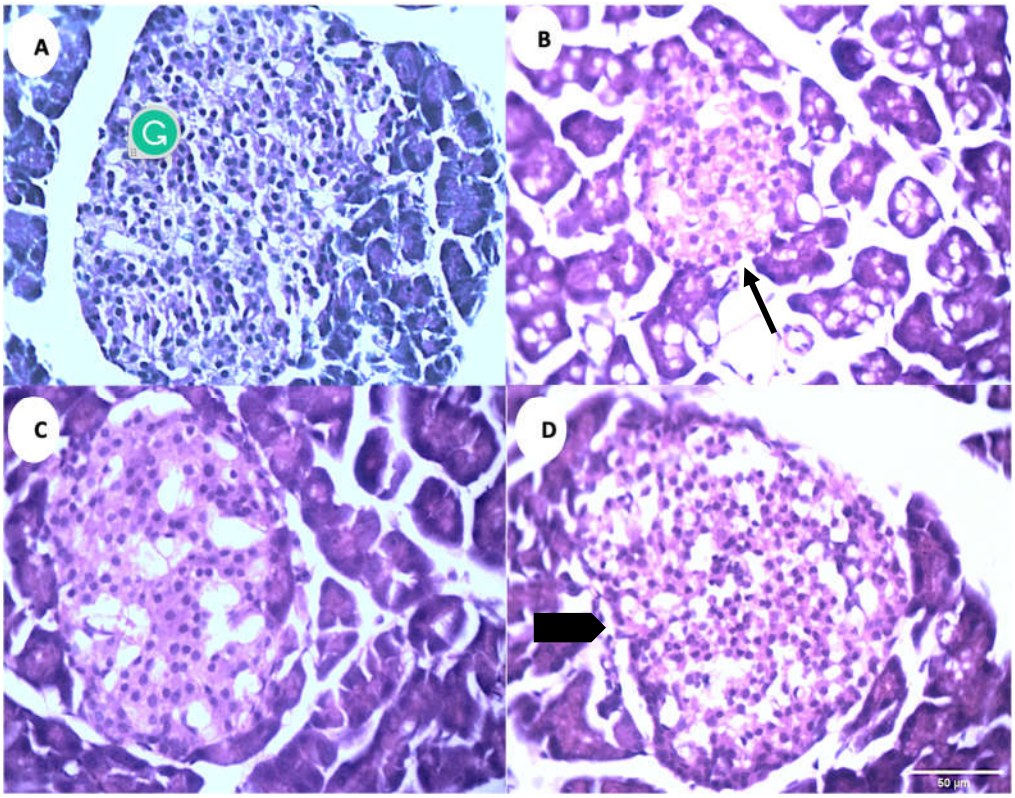

Figure 8. Representative photomicrographs of the islets of Langerhans in different treatment groups. **A.** Negative control group (NC). **B.** Positive control group (PC). **C.** Treatment group 1 (GS1). **D.** Treatment group 2 (GS2). Note the reduced diameter of islet in the PC group (thin arrow) and the increase in the islet diameter in the GS2 treatment group (Block arrow).

The microscopic analysis of the liver parenchyma in different treatment groups revealed increased sinusoidal and perisinusoidal space in the positive control group (PC) as compared to the negative control group (NC) (Fig. 9 A, B). Both treatment groups exhibit decreased sinusoidal and perisinusoidal space, but in treatment group 1 (GS1), the decrease is absent near the central vein, while in treatment group 2 (GS2), a more uniform decrease in the sinusoidal space is observed, including the area surrounding the central vein (Fig. 9 C, D).

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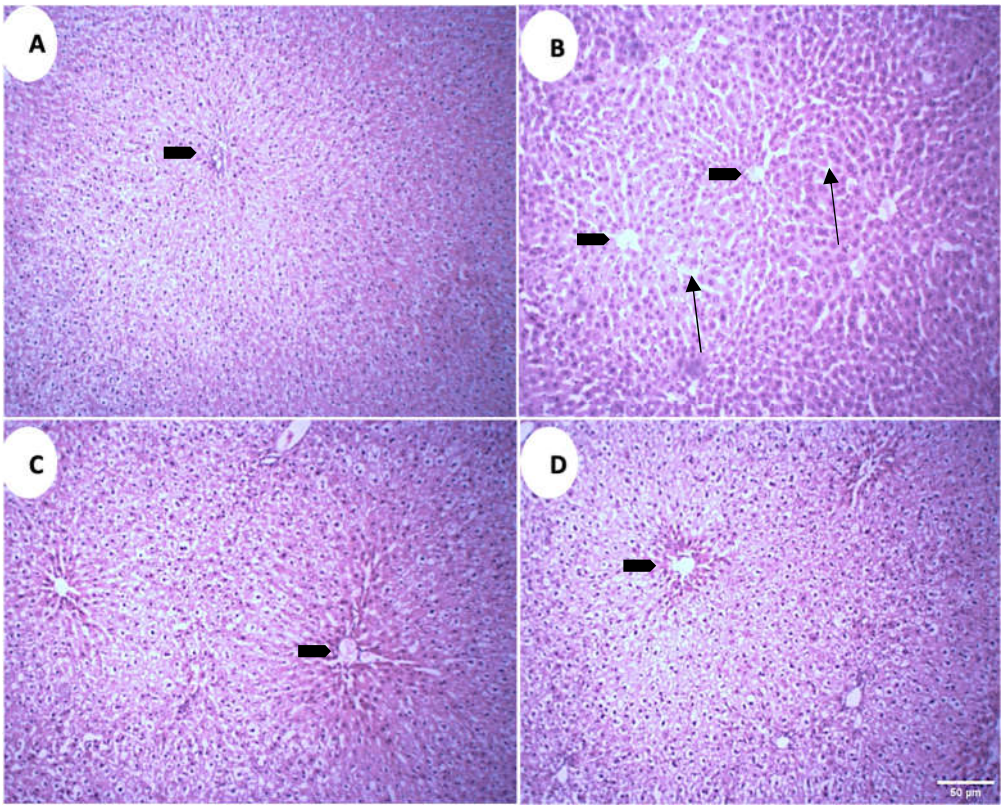

Figure 9. Representative photomicrographs of the liver parenchyma in different treatment groups. **A.** Negative control group (NC). **B.** Positive control group. **C.** Treatment group 1 (GS1). **D.** Treatment group 2 (GS2). Note the increase in the sinusoidal and perisinusoidal pace in the positive control group (PC) (Arrows). Block arrow: central vein.

4. Discussion:

The present study examined the effect of GS-dried crushed leaves on different metabolic parameters in a hyperglycemic rat model. The experimental diabetes was induced in the rats by using Alloxan. Alloxan is a β-cytotoxin that induces pancreatic β-cell damage, thus causing chemical diabetes [19]. The antioxidant ability of GS is due to the presence of many bioactive components in its leaves. These bioactive components are oleanane-type triterpenoid saponins and are known as anthraquinones, acidic glycosides, gymnemic acids alkaloids, and their products [18,20]. *In-vitro* Hepatoprotective activity of GS has also been reported previously [21-23]. Previously it has been reported that GS stimulates insulin release *in vitro* by increasing the membrane permeability of pancreatic beta cells [24-26]. GS treatment has been reported previously to modulate incretin activity, thereby triggering insulin release [18].

In the current study, we report an *in-vivo* effect of GS treatment on plasma insulin levels and an increase in insulin gene transcription. We speculate that the initial increase in the plasma insulin levels might be due to the change in the membrane permeability of beta cells induced by incretins resulting in insulin secretion and not synthesis. Secondly, the incretins are also responsible for the transcriptional activation of *Pdx1*. [27-29]. Incretins, along with insulin, induced an increased expression of *Pdx1*, as insulin is also responsible for increased transcriptional activity of *Pdx1* [30]. Increased *Pdx1* transcription resulted in further enhancement of *Ins-1* expression and insulin synthesis, depicted by a gradual increase in the insulin concentration in the treatment groups. Significant antioxidant activity of GS supplementation was observed in the present study in terms of the plasma levels of total oxidant status and hepatic enzymes like Paraoxonase, Arylesterase, AST, and ALT. Paraoxonase and arylesterase are associated with high-density lipoproteins (HDLs) and inhibit the oxidation of low-density lipoproteins (LDL) [31,32].

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Hyperglycemia is associated with elevated levels of AST and ALT [33]. Plasma albumin levels were raised in both of the treatment groups as plasma albumin levels are inversely associated with insulin reserves [34]. Superoxide dismutase (Sod1, Sod2) and catalase (Cat) are potent antioxidant enzymes and regulate the levels of reactive oxygen species (ROS) generated through hyperglycemia [35, 36]. Gene expression levels of these enzymes were significantly enhanced in both treatment groups. Nuclear factor erythroid 2-related factor (Nrf2), a transcription factor that mediates the redox homeostatic gene regulatory network and regulates the expression of *Sod1, Sod2,* and *Cat* expression and helps restore redox homeostasis [37]. NF-kB is an inflammation-sensitive transcription factor that initiates the cytokine response during inflammation [38]. A significant decrease in the mRNA expression levels of antioxidant enzymes like *Sod1, Sod2, Cat,* and *Nrf2* expression was present in the positive control group, followed by a gradual and significant increase in both treatment groups. Malondialdehyde (MDA) is a marker of lipid peroxidation and is associated with hyperglycemic-induced dyslipidemia [39]. MDA levels in the positive control group were increased non-significantly and followed by a reduction in both treatment groups.

Insulin receptor substrates (Irs1, Irs2) in hepatocytes are activated by insulin receptor [40]. Insulin receptor proteins are also responsible for the transcriptional upregulation of the hepatic glucokinase (Gck) gene and subsequent carbohydrate metabolism in the hepatocytes [41]. Irs1 has a constant hepatic expression, whereas Irs2 is only expressed during fasting as Irs2 is inhibited by insulin [42-44]. Hepatic glucokinase (Gck) expression also depends on feeding and fasted state and is primarily upregulated by insulin and downregulated by glucagon [45]. In the present study, the hepatic expression of *Irs-1* and *Gck* was reduced, and that of *Irs2* was increased in the positive control group because of low plasma insulin levels. Both treatment groups exhibited a marked increase in *Irs1* and *Gck* and a decline in *Irs2* levels owing to increased plasma insulin levels. Hepatic expression of insulin-sensitive Forkhead family of transcription factors (FOXOs) has been shown to increase during fasting because of a lack of insulin [46]. FoxO1 are involved in the hepatic production of glucose and VLDL in the absence of indulin, as increased hepatic insulin signaling has been shown to inhibit FoxO1 and thereby reduced the release of glucose and VLDL from the liver by restraining microsomal triglyceride transfer protein (MTP) and apolipoprotein B (ApoB) expression [47-49]. FoxA2 also stimulates hepatic expression of *MTB*, thereby enhancing VLDL release from the liver [50]. FoxA2 is deactivated/phophorylated by insulin resulting in decreased production of MTB and VLDL from the liver [51]. Recently Foxk1 has been shown to be positively regulated by insulin and results in the enahncement of glucose metabolism by hepatocytes [52]. In the present study we speculate that the primary pathway through which GS supplementation improves dyslipidemia in hyperglycemic rats is mainly through enhanced hepatic signaling of insulin. Sterol regulatory binding protein 1c (SREBP1c) is a transcription factor protein that enhances the expression of key hepatic enzymes involved in fatty acid synthesis [53]. SREBP1c activation requires high physiological levels of insulin [54]. Carbohydrate response element binding protein (ChREBP) is a transcription factor activated by glucose metabolites and, in turn, enhances hepatic lipogenesis by upregulating hepatic lipogenic enzymes [55, 56]. In the present study, the GS supplementation significantly enhanced SREBP1c and significantly reduced ChREBP mRNA expression in the liver, primarily through enhanced insulin and reduced plasma glucose levels. In conclusion, the present study suggests that *Gymnema Sylvestre* leaves, in their crude form, can act as antioxidative, hepatoprotective, and hypocholesterolemic agents in an experimentally induced diabetic rat model.

Supplementary Materials: The data is contained within the article.

Author Contributions: "Conceptualization, S.I. and H.A.; methodology, H.M, I.Q., M.B.; writing—original draft preparation, H.M., S.I.; writing—review and editing, S.I., F.J., H.A. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest: "The authors declare no conflict of interest."

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