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# Determination of Pharmacological Drug Interaction of Glibenclamide and *Hippophae rhamnoides* in Streptozotocin (STZ) Induced Diabetes Mellitus in Rat Model

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**Keywords:** Drug interaction, *Hippophae rhamnoides*, Glibenclamide, Diabetes Mellitus, Antioxidant activity, Male Albino Wistar Rats.

**ABSTRACT**

The concomitant use of *Hippophae rhamnoides* berry powder and GLB is a possible therapy for diabetic patients. In this study, the pharmacological drug interaction of *H. rhamnoides* with GLB was assessed using a Streptozotocin-induced diabetes mellitus rat model. Male Albino Wistar rats were divided into 5 groups, consisting of 10 rats in each group. Normal rats in group 1 were treated with 1% CMC. The diabetic rats in groups 2 to 5 were given vehicle, GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and combination of *H. rhamnoides* and GLB, respectively. All treatments were administered once daily for 21 days by oral route. In diabetic rats, a combination of *H. rhamnoides* and GLB significantly improved blood glucose levels and body weight compared to *H. rhamnoides* and GLB alone. The lipid profile, including serum TC, TG, HDL-C, and LDL-C was found to be significant in diabetic rats treated with a combination of *H. rhamnoides* and GLB when compared to diabetic groups exposed to a single treatment of *H. rhamnoides*. Additionally, the beneficial effects of combination therapy on antioxidant parameters in diabetic rats were found to be greater than those of *H. rhamnoides* and GLB monotherapy. Moreover, the combination of *H. rhamnoides* and GLB had the greatest restoration effect on pancreatic and liver tissues. Based on the results obtained from the current study, it can be concluded that the combined effect of *H. rhamnoides* and GLB is found to be efficacious as compared to individual therapy of *H. rhamnoides* and GLB.

## 1. INTRODUCTION

Diabetes is a complex disease with numerous causes and pathophysiology. The International Diabetes Federation projects that approximately 74 million people are living with diabetes in India, and this number is expected to rise to 125 million by 2045.[1] Diabetes mellitus (DM) is a chronic metabolic disorder caused by disruptions in insulin production (which regulates blood sugar) as well as carbohydrate, protein, and lipid metabolism. Hyperglycaemia in diabetes mellitus causes substantial nerve and blood vessel damage and is linked to a variety of complications such as retinopathy, microangiopathy, and nephropathy.[2]

Diabetes mellitus is divided into two main types: Type 1 and Type 2. Type 1 diabetes mellitus (T1DM), also known as insulin-dependent diabetes mellitus (IDDM), is caused by impaired insulin production, whereas type 2 diabetes mellitus (T2DM) is associated with the inability of cells to respond to insulin, resulting in insulin resistance, and is thus known as non-insulin dependent diabetes mellitus (NIDDM). There are a great variety of accessible synthetic or semi-synthetic anti-diabetic medicines that lower blood sugar levels but are associated with serious side effects. To obtain better treatment with fewer adverse effects, the search for alternative antidiabetic drugs from natural sources is used traditionally.[3]

Due to continuous and chronic hyperglycaemia, diabetics and experimental models experience severe oxidative stress, which depletes the activity of the antioxidative defence system and stimulates the formation of new free radicals.[4] Streptozotocin (STZ) is often used for the induction of diabetes mellitus in experimental animals. STZ results in the production of reactive oxygen species that cause oxidative damage.[3] Hence, scientific and public interest in antioxidant theory has been increasing over the last few decades. As a result, in addition to blood glucose control, oxidative stress management provides another avenue for disease treatment. Drugs with antioxidant and free radical scavenging properties may aid in beta cell regeneration and protect pancreatic islets from the cytotoxic effects of STZ.[5]

Glibenclamide (GLB) is most frequently prescribed oral hypoglycaemic agent.[6] GLB is a sulphonyl urea used as an oral hypoglycaemic agent that is commonly used to treat type 2 diabetes. It is known to promote insulin secretion through inhibition of ATP-sensitive $K^+$ channels in the pancreatic cells.[7]

***Hippophae rhamnoides***

*H. rhamnoides* commonly known as Sea buckthorn (Family- Elaegnaceae) is a thorny deciduous shrub local to several countries of Europe and Asia. *H. rhamnoides* is highly recognized for its antioxidant, cardioprotective, antiatherogenic, antidiabetic, hepatoprotective, anti-carcinogenic, immunomodulatory, antiviral, antibacterial, anti-inflammatory and vasorelaxant effects. The berries provide abundant amounts of vitamin C and E, carotenoids, mainly ß-carotene, lycopene, lutein, and zeaxanthin. It is a good source of flavonoids too, mainly quercetin, kaempferol, myricetin, and isorhamnetin, and an important source of tocopherols. *H. rhamnoides* is a very famous shrub and has been used as herbal medicine for many years, not only as therapeutic but also as prophylactic and health-promotional mediators. The major constituents of *H. rhamnoides*, which are accountable for the antidiabetic effect, are flavonoids, phenolic compounds, carotenoids, tannins and phytosterol, which act by achieving reduced blood glucose concentrations by dietary supplementation with sea buckthorn.[8] Oxidative stress is also the major cause of hyperglycaemia. Antioxidant property of *H. rhamnoides* supplies endogenous defence systems and reduce both initiation and propagation of reactive oxygen species.[9]

**Drug Interactions**

When two or more drugs react with each other, it is called a drug-drug interaction. Herb-drug interactions are drug interactions that occur between herbal medicines and allopathic drugs. These types of interactions are more common than drug-drug interactions because herbal drugs often contain multiple pharmacologically active ingredients, while conventional drugs typically contain only one.[10]

As people often take different herbs in combination with prescribed modern medication, there is a potential for interaction. Interactions between herb and drug may increase or decrease the pharmacological or toxicological effects of either component. Since the combination of GLB and *H. rhamnoides* also has the potential to be employed by diabetic patients, the study was being planned to determine the pharmacological drug interaction between *H. rhamnoides* and GLB in STZ-induced type 2 diabetic rats.

## 2. MATERIAL AND METHODS

### 2.1. Purchase and Authentication of Marketed Powder

The powder of *H. rhamnoides* berries was procured from Agasthiya organics, Karnataka, India. The drug powder sample was subjected to identification and authentication by the Alarsin organization, Andheri(E), Mumbai.

### 2.2. Preparation of Study Drugs

- **Preparation of *Hippophae rhamnoides***

The marketed powder of *Hippophae rhamnoides* berries was suspended in 1% CMC solution and was administered orally to experimental animals.

- **Preparation of GLB**

The powder of GLB was suspended in 1% CMC solution and was administered orally to experimental animals.

### 2.3. Experimental Animal

Albino Male Wistar rats (n=50) weighing 200-250gm, were procured for experimental study. Animals were housed in polypropylene cages with corn cob bedding and access to animals laboratory feed with the *ad.-libitum* water. The animal house was maintained at an average temperature (24°C± 2°C) and 40-70 % RH, with 12 h light-dark cycle. All animals were allowed to acclimatize for 7 days before experimentation. The experiment was conducted according to the Protocol No. BVCP/IAEC/06/2020 approved by IAEC in accordance with the guidelines set by the CPCSEA. The study was carried out in an animal house (Reg. no. 762/PO/Re/S/03/CPCSEA), Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Navi Mumbai.

### 2.4. Induction of Diabetes

For diabetes induction, Streptozotocin (STZ) 45mg/kg[11] freshly prepared citrate buffer, was injected (i.p.) to the overnight fasted Wistar rats after an acclimatization period of 7 days. The blood glucose levels were estimated using tail vein method before and after the induction of STZ. Hyperglycaemia was confirmed by the raised blood-glucose levels determined on day 3

and then on day 7 after induction. The rats with blood glucose levels greater than 250mg/dl were considered diabetic and were used for the study.

**2.5. Experimental Design**

Rats were divided into 5 groups consisting of 10 rats in each group. All treatments were administered orally, once daily using oral gavage needle for a period of 21 days.

**Group 1- Vehicle Control Group**

Normal rats received 1% Carboxymethylcellulose (CMC)

**Group 2- Disease Control Group**

STZ-diabetic rats received 1% Carboxymethylcellulose (CMC)

**Group 3- GLB Group**

STZ-diabetic rats treated with GLB (5 mg/kg bodyweight) [11]

**Group 4- *H. rhamnoides* Group**

STZ-diabetic rats treated with *H. rhamnoides* (400mg/kg bodyweight) [12]

**Group 5- *H. rhamnoides*+ GLB Group**

STZ-diabetic rats treated with *H. rhamnoides* (400mg/kg bodyweight) + GLB (5mg/kg bodyweight)

**2.6. Measurement of Body Weight**

The body weight of the animals was measured twice in a week from the day of procurement to the end of the experiment.

**2.7. Evaluation of Blood Glucose Levels**

Blood glucose levels were checked on days 1, 7, 14 and 21 by glucometer (Dr. Morepen Gluco-one).

**2.8. Lipid Profile Estimation**

On 21st Day, Blood samples were withdrawn from the retro-orbital venous plexus under mild ketamine anaesthesia from the overnight fasted animals into Eppendorf tubes. The serum was

separated by cold centrifugation at 4000 rpm for 15 min. and used for the evaluation of Serum parameters such as Serum Total Cholesterol (TC), Triglycerides (TG), High-density lipoprotein-cholesterol (HDL-C) and Low-density lipoprotein cholesterol (LDL-C). All these parameters were estimated by spectrophotometric method using commercially available standard kit supplied by ERBA Diagnostic Mannheim GmbH, Germany and ERBA autoanalyzer.

### 2.9. Histopathological Evaluation

At the end of the experimental period i.e. on 22$^{nd}$ day, all animals were sacrificed using overdose of $CO_2$. Pancreas and livers were dissected immediately, washed in cold saline (0.9% NaCl), and preserved in 10% formalin and sent for histopathological evaluation.

### 2.10. Evaluation of Anti-oxidant activity

For the *In-vivo* enzyme assay, immediately after sacrificing animals, the liver, pancreas and kidney were separated, washed with cold saline. A portion of these washed pancreas, liver and kidney were minced and homogenized (REMI) using phosphate buffer (pH 7.4) and was used for the evaluation of antioxidant parameters. The measured parameters included the activities of MDA (Method of Buege& Aust, 1978)[13]and catalase (Method of Aebi, 1984).[14]

### 2.11. Statistical analysis

The results were expressed as mean ± Standard Error of Mean (SEM) for n=6 animals. The statistical analysis of the results was carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test. Difference was considered significant at $p< 0.05$, using Graph Pad Prism software (version 9.3.1).

## 3. RESULTS AND DISCUSSION

### 3.1. Effect on Body Weight

Weight loss is a common symptom of diabetes mellitus. In this study, disease control rats showed a significant ($P < 0.05$) decrease in their weight throughout the experiment as compared to all treatment group rats. At the end of the treatment study, the body weight of the rats in GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and combination of *H. rhamnoides* with GLB treated groups, increased significantly ($P < 0.05$); compared with the body weight of disease control group. (Table no. 1, Fig. no. 1).

**Table No. 1: Effect of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Body Weight (g) in STZ induced diabetes mellitus in Wistar rats. (Mean±SEM)**

| Groups | Body Weight (g) | | | | |
|---|---|---|---|---|---|
| | **Normal** | **Day0** | **Day7** | **Day14** | **Day21** |
| **VehicleControl** | 271±1.05 | 285±2.04 | 297±0.79 | 313±1.03 | 327±1.09 |
| **Disease control** | 277±1.27 | 234±1.26 | 203±1.22 | 175±1.19 | 153±0.88 |
| **GLB** | 274±1.39 | 238±1.47 | 273±1.17[a] | 302±0.98[a] | 321±1.01[a] |
| ***H. rhamnoides*** | 272±2.65 | 230±2.30 | 267±1.52[a] | 300±2.40[a] | 315±1.62[a,b] |
| ***H. rhamnoides* +GLB** | 278±1.70 | 231±1.07 | 277±2.77[a,d] | 306±1.74[a,c,d] | 319±1.33[a,d] |

All the values are expressed as Mean ±SEM for n=6 animals.

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

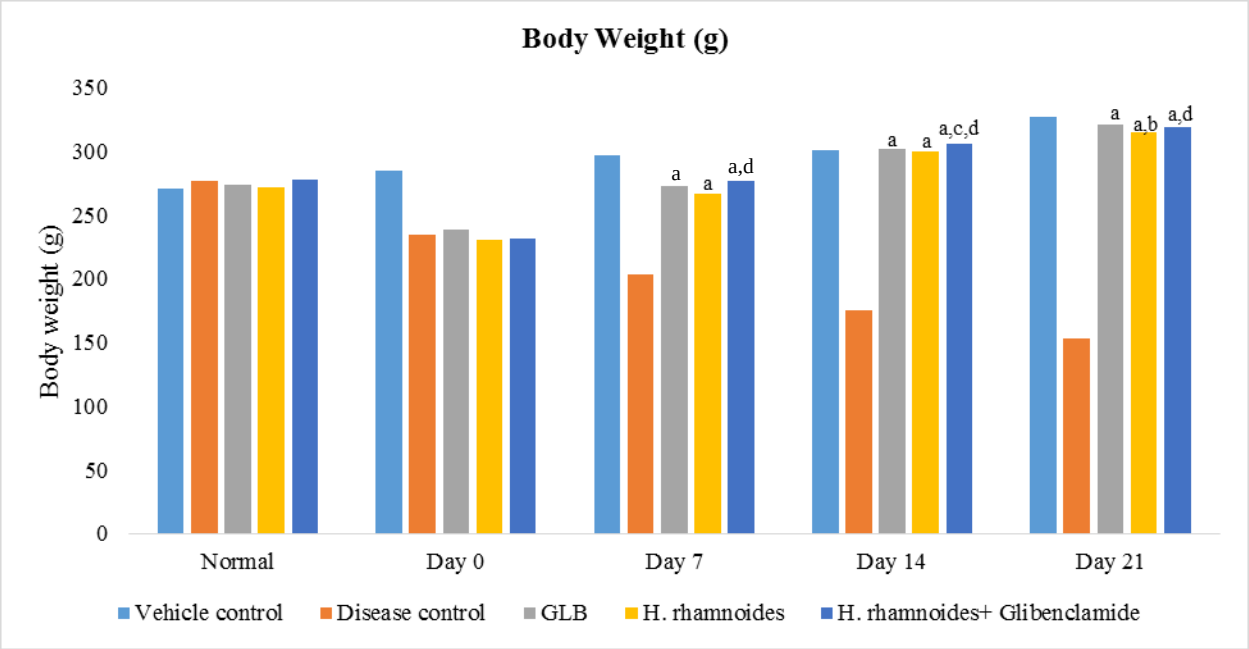



**Fig. No. 1: Effect of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Body Weight (g) in STZ induced diabetes mellitus in Wistar rats.**

All the values are expressed as Mean ±SEM for n=6 animals.

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

### 3.2. Effect on Blood Glucose Level

All treatment groups (GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and combination of *H. rhamnoides*+ GLB) were found efficacious in reducing elevated blood glucose levels over 21days in STZ-induced diabetic rats. Blood glucose levels were reduced significantly (P <

0.05) on day 14 and day 21 of treatment when compared with disease control group. The combination group of *H. rhamnoides* with GLB showed significant (P < 0.05) effect from day 7, hence showed better results than single treatment of GLB and *H. rhamnoides.* (Table no. 2, Fig. no. 2).

**Table No. 2: Effect of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Blood glucose levels (mg/dl) in STZ induced diabetes mellitus in Wistar rats. (Mean±SEM)**

| Groups | Blood glucose level(mg/dl) | | | | |
|---|---|---|---|---|---|
| | Normal | Day0 | Day7 | Day14 | Day21 |
| **Vehicle Control** | 115±1.49 | 116±2.07 | 118±1.76 | 128±1.74 | 119±2.67 |
| **Disease control** | 117±1.98 | 503±17.60 | 526±23.81 | 550±5.38 | 543±17.70 |
| **GLB** | 117±1.24 | 517±2.87 | 434±3.11[a] | 288±1.99[a] | 192±6.56[a] |
| ***H. rhamnoides*** | 116±1.87 | 514±1.43 | 440±6.54[a] | 359±1.47[a] | 140±2.20[a] |
| ***H. rhamnoides* +GLB** | 115±1.70 | 517±1.29 | 386±3.29[a,c,d] | 196±2.35[a,c,d] | 117±1.40[a,c,d] |

All the values are expressed as Mean ±SEM for n=6 animals

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

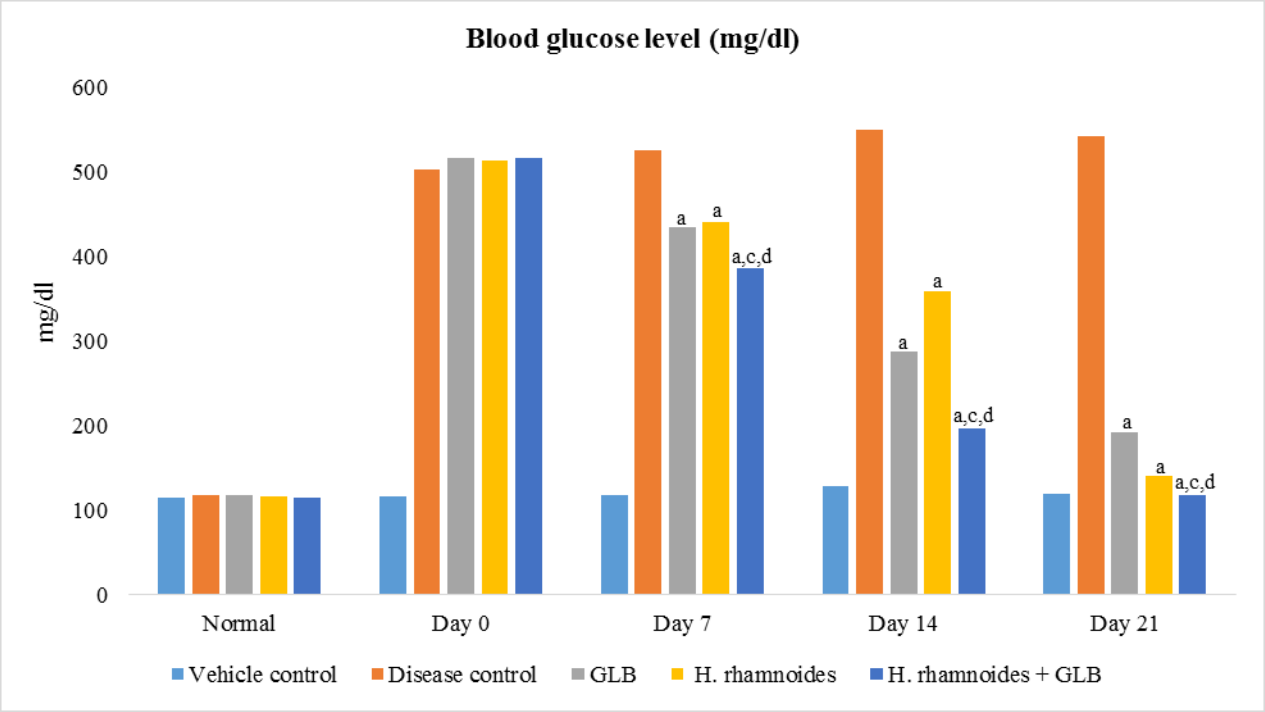



**Fig. no. 2 Effect of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Blood glucose levels (mg/dl) in STZ induced diabetes mellitus in Wistar rats.**

All the values are expressed as Mean ±SEM for n=6 animals.

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

### 3.3. Effect on Lipid Profile

Diabetes is linked to a hyperlipidemia. It's a well-known fact that poor glucose control in

diabetes causes changes in the blood lipid profile.[4] GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and combination of *H. rhamnoides* with GLB treated groups showed significant ($P < 0.05$) decrease in the lipid levels of TC, TG, LDL-C and VLDL-C in STZ-induced diabetic rats compared to the disease control group. HDL-C was significantly increased in all treatment groups when compared to disease control group. (Table No. 3, Fig. No. 3)

**Table No. 3: Effect of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on lipid profile (mg/dl) in blood serum of STZ induced diabetes mellitus in Wistar rats. (Mean±SEM)**

| Groups | Lipid Profile (mg/dl) | | | | |
|---|---|---|---|---|---|
| | TC | TG | HDL-C | LDL-C | VLDL-C |
| **Vehicle Control** | 82.3± 0.40 | 45.66±0.32 | 49.22±0.29 | 24.00± 0.60 | 9.132±0.06 |
| **Disease control** | 125.0±0.43 | 81.55±0.38 | 17.39±0.16 | 91.30±0.49 | 16.31±0.07 |
| **GLB** | 83.21±0.25[a] | 54.69±0.29[a] | 33.50±0.34[a] | 38.80±0.53[a] | 10.94±0.05[a] |
| ***H. rhamnoides*** | 90.92±0.19[a] | 59.48±0.77[a] | 22.00±0.28[a] | 57.00±0.27[a] | 11.90±0.15[a] |
| ***H. rhamnoides* +GLB** | 89.48±0.28[a] | 55.05±0.52[a,d] | 33.56±0.20[a,d] | 44.90±0.50[a,d] | 11.01±0.10[a,d] |

All the values are expressed as Mean ±SEM for n=6 animals

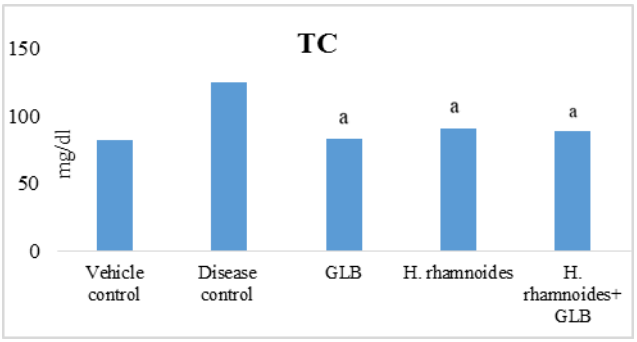
**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at $p< 0.05$.**

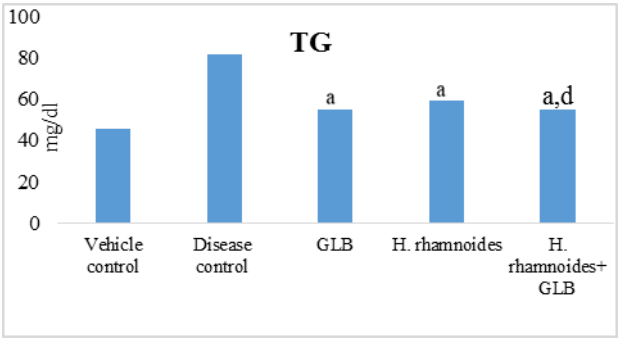
a = $P < 0.05$, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = $P < 0.05$, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = $P < 0.05$, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = $P < 0.05$, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).


TC
150
100
50
0
mg/dl
a
a
a
Vehicle control
Disease control
GLB
H. rhamnoides
H. rhamnoides+ GLB


TG
100
80
60
40
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mg/dl
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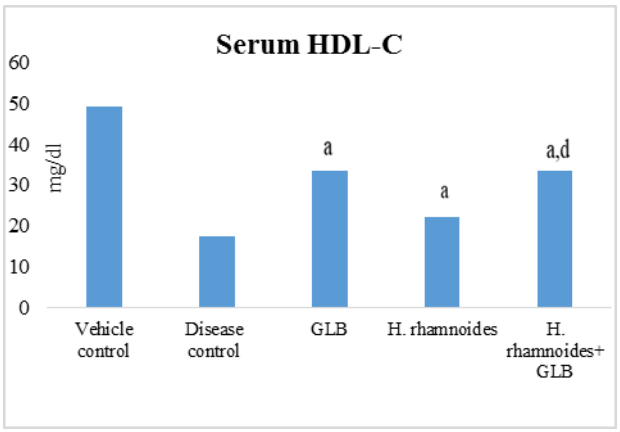
a,d
Vehicle control
Disease control
GLB
H. rhamnoides
H. rhamnoides+ GLB


Serum HDL-C
60
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30
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mg/dl
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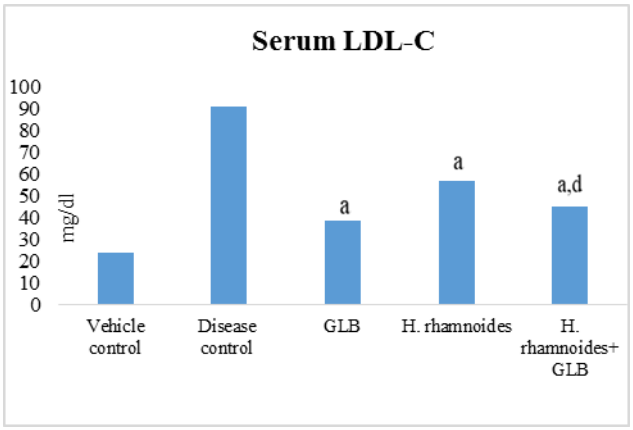
a,d
Vehicle control
Disease control
GLB
H. rhamnoides
H. rhamnoides+ GLB


Serum LDL-C
100
90
80
70
60
50
40
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mg/dl
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Vehicle control
Disease control
GLB
H. rhamnoides
H. rhamnoides+ GLB

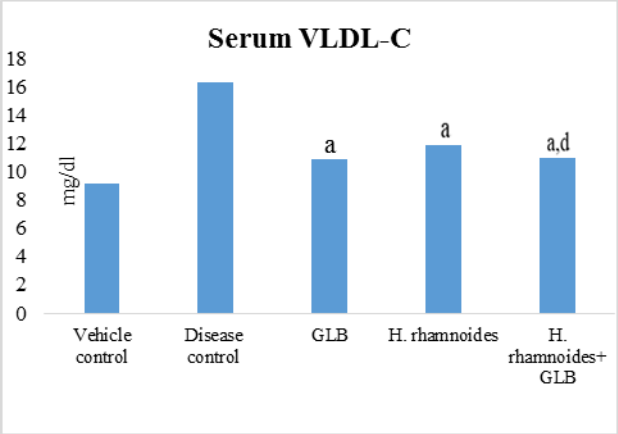



**Fig. No. 3: Effect of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Serum-TC, Serum-TG, Serum HDL-C, Serum LDL-C and Serum VLDL-C (mg/dl) in blood serum of STZ induced diabetes mellitus in Wistar rats**

All the values are expressed as Mean ±SEM for n=6 animals.

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

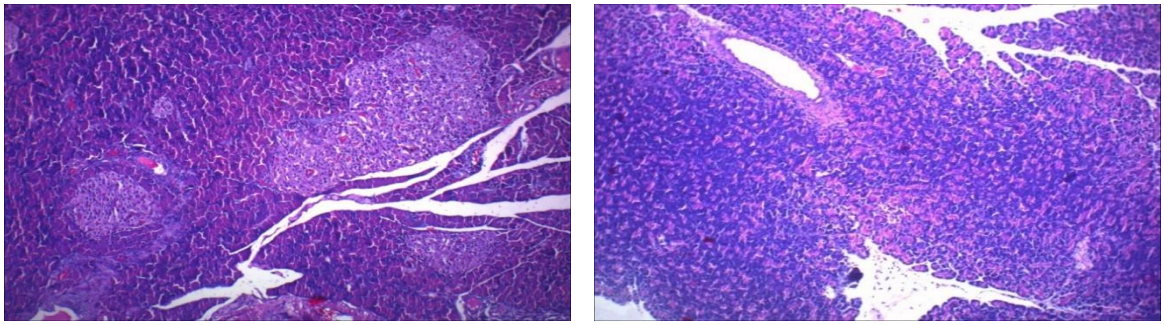
b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

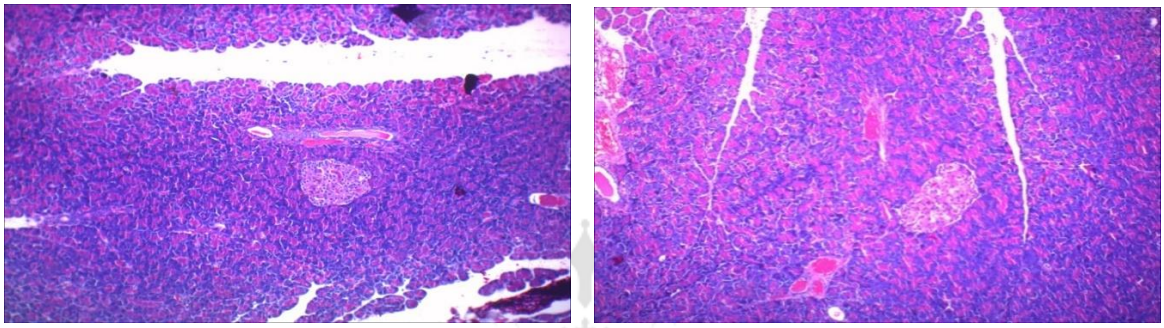
d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

### 3.4. Histopathological Evaluation of Isolated Livers and Pancreas

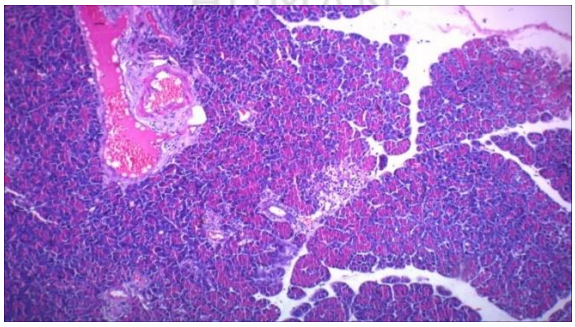
#### 3.4.1. Histopathological evaluation of Pancreas in STZ induced diabetic rats



**A. Vehicle Control** **B. Disease control (STZ-45mg/kg)**



**C. GLB (5 mg/kg)** **D. *H. rhamnoides* (400mg/kg)**



**E. *H. rhamnoides* +GLB**

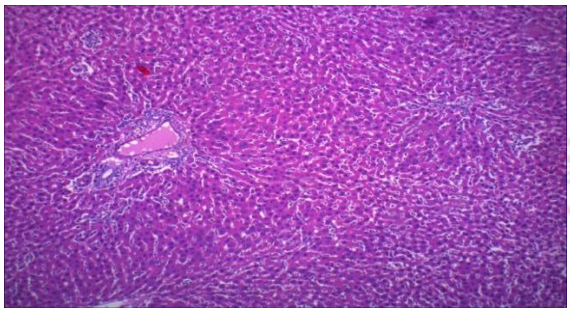
**Fig. No. 4: The histopathological findings of the Pancreas 100X magnification**

(A) Vehicle control group – No abnormality detected in pancreatic islets. (B) Disease control group – moderate decrease in the no. of pancreatic islets. (C) GLB-treated group - minimal decrease in the no. of pancreatic islets. (D) *H. rhamnoides* group – showing mild decrease in the number of the islets of the pancreas with mild vacuolization. (E) *H. rhamnoides* + GLB group - minimal decrease in the no. of pancreatic islets.

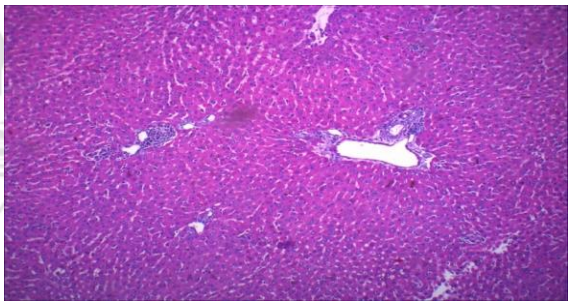
**Table No. 4: Effects of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Pancreas histopathology in STZ induced diabetes mellitus in Wistar ratsat magnification 100X resolution.**

| Groups | Microscopic observation |
|---|---|
| **Vehicle Control** | No abnormality detected in pancreatic islets. |
| **Disease control** | Moderate decrease in the number of pancreatic islets. |
| **GLB** | Minimal decrease in the number of pancreatic islets. |
| ***H. rhamnoides*** | Mild decrease in the number of the islets of the pancreas with mild vacuolization. |
| ***H. rhamnoides*** **+GLB** | Minimal decrease in the number pancreatic islets. |

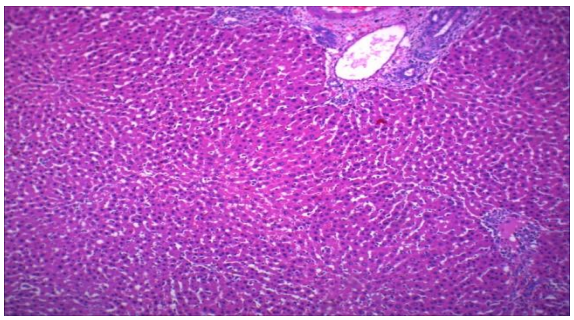
### 3.4.2. Histopathological evaluation of Liver in STZ induced diabetic rats



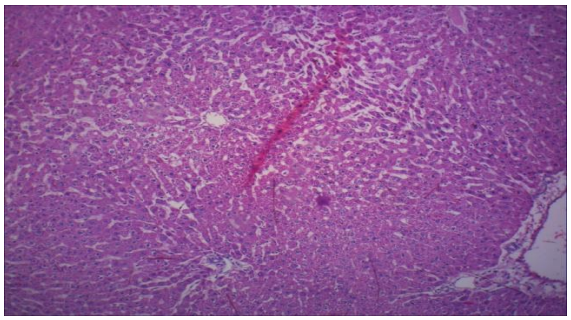
**A. Vehicle Control**



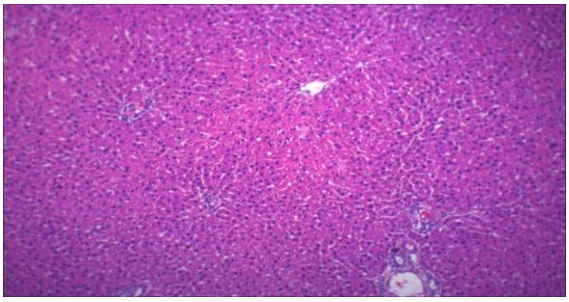
**B. Disease control (STZ-45mg/kg)**



**C. GLB (5 mg/kg)**



**D. *H. rhamnoides* (400mg/kg)**



**E. *H. rhamnoides* +GLB**

**Fig. No. 5: The histopathological findings of the Liver 100X magnification**

(A) Vehicle control group – No abnormality detected. (B) Disease control group – Moderate reduction in sinusoidal spaces, hepatocyte hypertrophy and lymphoid infiltration. (C) GLB-treated group - Minimal reduction in sinusoidal spaces, hepatocyte hypertrophy and lymphoid infiltration. (D) *H. rhamnoides* group – Mild reduction in sinusoidal spaces, hepatocyte hypertrophy and glycogen deposition. (E) *H. rhamnoides* + GLB group - Minimal reduction in sinusoidal spaces, hepatocyte hypertrophy and glycogen deposition.

**Table No. 5: Effects of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Livers histopathology in STZ induced diabetes mellitus in Wistar rats at magnification 100X resolution.**

| **Groups** | **Microscopic observation** |
|---|---|
| **VehicleControl** | No abnormality detected. |
| **Disease control** | Moderate reduction in sinusoidal spaces, hepatocyte hypertrophy and lymphoid infiltration. |
| **GLB** | Minimal reduction in sinusoidal spaces, hepatocyte hypertrophy and lymphoid infiltration. |
| ***H. rhamnoides*** | Mild reduction in sinusoidal spaces, hepatocyte hypertrophy and glycogen deposition. |
| ***H. rhamnoides* +GLB** | Minimal reduction in sinusoidal spaces, hepatocyte hypertrophy and glycogen deposition. |

### 3.5. Evaluation of Anti-oxidant Activity

#### 3.5.1. Effect on MDA Levels

All treatment groups (GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and combination of *H. rhamnoides* with GLB) showed significant ($P < 0.05$) reduction in the levels of MDA in the tissue homogenates of liver, kidney and pancreas of STZ-induced diabetic rats as compared to the disease control group.

**Table No. 6: Effects of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on MDA level (µmol/g weight of tissue) in liver, kidney and pancreas of STZ induced diabetes mellitus in Wistar rats. (Mean±SEM)**

| **Groups** | **MDA level (µmol/g weight of tissue)** | | |
|---|---|---|---|
| | MDA(Liver) | MDA(Kidney) | MDA(Pancreas) |
| **Vehicle Control** | 1.850±0.050 | 3.061±0.039 | 3.039±0.027 |
| **Disease control** | 4.740±0.036 | 5.937±0.042 | 6.209±0.129 |
| **GLB** | 1.939±0.032[a] | 3.312±0.036[a] | 3.257±0.039[a] |
| ***H. rhamnoides*** | 2.854±0.078[a] | 3.529±0.037[a] | 2.930±0.031[a,b] |
| ***H. rhamnoides* +GLB** | 2.386±0.085[a,d] | 2.560±0.123[a,c,d] | 2.734±0.057[a,c,d] |

All the values are expressed as Mean ±SEM for n=6 animals.

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = $P < 0.05$, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = $P < 0.05$, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = $P < 0.05$, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = $P < 0.05$, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

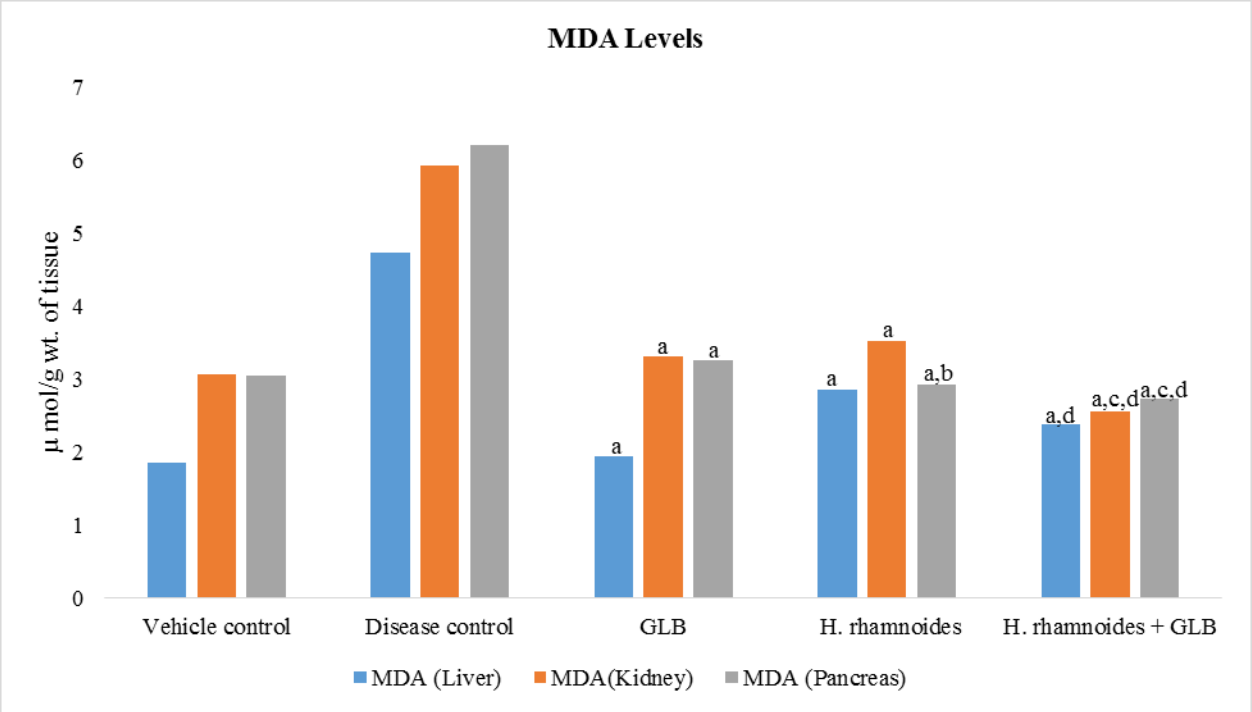



**Fig. No. 6: Effects of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on MDA level (µmol/g weight of tissue) in liver, kidney and pancreas of STZ induced diabetes mellitus in Wistar rats.**

All the values are expressed as Mean ±SEM for n=6 animals.

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

### 3.5.2. Effect on Catalase Levels

All treatment groups (GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and combination of *H. rhamnoides* with GLB) showed significant (P < 0.05) increase in the levels of Catalase in the tissue homogenates of liver, kidneyand pancreas of STZ-induced diabetic rats as compared to that of disease control group.

**Table No. 7: Effects of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Catalase level (µmol/g weight of tissue) in liver, kidney and pancreas of STZ induced diabetes mellitus in Wistar rats. (Mean±SEM)**

| Groups | Catalase level (µmol/g weight of tissue) | | |
|---|---|---|---|
| | Catalase (Liver) | Catalase (Kidney) | Catalase (Pancreas) |
| **Vehicle Control** | 81.59±1.28 | 72.12±2.10 | 72.89±3.28 |
| **Disease control** | 45.37±1.94 | 40.56±1.23 | 40.16±2.69 |
| **GLB** | 89.18±1.10[a] | 83.77±1.15[a] | 84.51±0.95[a] |
| ***H. rhamnoides*** | 73.83±0.70[a] | 83.29 ±0.54[a] | 76.91±0.46[a] |
| ***H. rhamnoides* +GLB** | 82.22±1.29[a,d] | 86.99±0.19[a] | 85.21±0.44[a,d] |

All the values are expressed as Mean ±SEM for n=6 animals

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

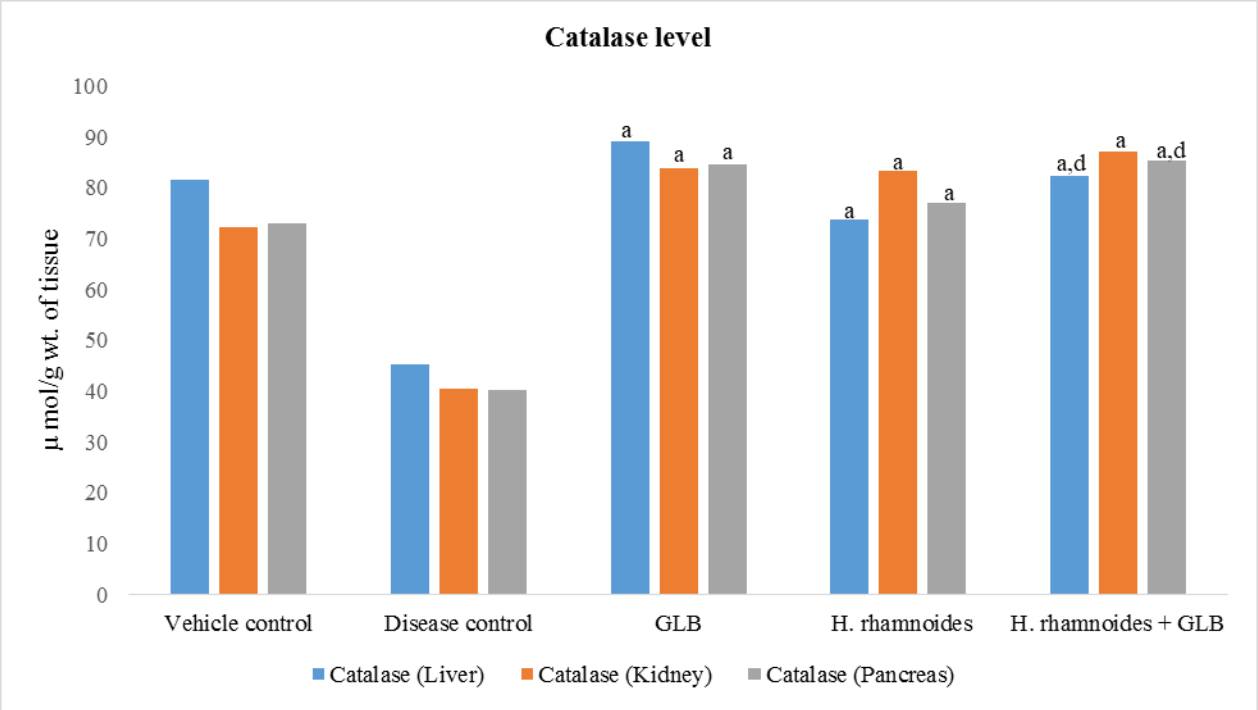



**Fig. No. 7: Effects of drug GLB (5 mg/kg), *H. rhamnoides* (400 mg/kg) and their combination on Catalase level (µmol/g weight of tissue) in kidneys in STZ induced diabetes mellitus in Wistar rats.**

All the values are expressed as Mean ±SEM for n=6 animals

**The statistical analysis of the results were carried out using one way ANOVA followed by Tukey's multiple comparison post hoc test at p< 0.05.**

a = P < 0.05, All treatments Groups, statistically significant when compared with Group 2 (Disease control).

b = P < 0.05, Group 4 (*H. rhamnoides*), statistically significant when compared with Group 3 (GLB).

c = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 3 (GLB).

d = P < 0.05, Group 5 (*H. rhamnoides* + GLB), statistically significant when compared with Group 4 (*H. rhamnoides*).

## DISCUSSION

Diabetes mellitus is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality.[15] Synthetic antidiabetic drugs, even when used in combination, fail to meet the demand for controlling diabetes-related complications, and these combinations may wind up being a source of numerous side effects.[16]

There are a great variety of accessible synthetic or semi-synthetic anti-diabetic medicines that lower blood sugar levels but are associated with serious side effects. To obtain better treatment with fewer adverse effects, the search for alternative antidiabetic drugs from natural sources is used traditionally. Therefore, Herbal medicines are gaining importance as they are cost-effective and also display improved therapeutic actions with lesser side effects. Many diabetic patients are known to take concomitant therapy of antidiabetic herbs in addition to their allopathic therapies. This may lead to an herb – drug interaction. Concomitant use of herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. Examples of clinical and experimental studies include- A) Aloe-vera with Glibenclamide (GLB) results in additive effect on lowering of blood glucose. B) Use of Karela with Metformin indicated significant decrease in serum glucose levels. And C) Combination of Sesame oil with GLB Improved anti-hyperglycaemic effect.[10]

Rats are widely used diabetes models, because they provide enough tissue for biochemical assays and can undergo concurrent physiologic procedures that are difficult to do in smaller animals. The majority of studies to date have used rats with insulin-deficient diabetes induced by STZ, which destroys pancreatic cells. The Wistar rat is one of the most frequently used laboratory animal.[17]

The production of reactive species that cause oxidative damage is linked to STZ-induced diabetes mellitus. Streptozotocin enters the pancreatic beta cell through glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ activates poly adenosine diphosphate ribosylation and nitric oxide production. STZ action results in the destruction of pancreatic beta cells by necrosis. The most common dose of STZ used to produce insulin-dependent diabetes in adult rats is 40-60 mg/kg, however larger doses are also employed. When tail blood glucose concentrations in fed rats are greater than 200–300 mg/dl two days after STZ injection, the rats are considered diabetic. In rats, type 2 diabetes

can be induced through i.v. (tail vein) or i.p. (intraperitoneal) therapy with STZ in the initial days of life.[18]

*H. rhamnoides* fruit has long been used in folk medicine to treat diabetes. The major constituents of *H. rhamnoides*, which are accountable for the antidiabetic effect, are flavonoids, phenolic compounds, carotenoids, tannins and phytosterols. Various mechanisms have been reported in the literature to explain the possible antidiabetic effects of *H. rhamnoides.* These mechanisms include reduction in blood glucose levels by increasing insulin secretion and insulin sensitivity.[13] Glibenclamide (5mg/kg), also referred to as glyburide, is commonly used Sulfonylurea for the treatment of type 2 diabetes mellitus. The drug is commonly used either as monotherapy or multiple drug therapy. The drug has been selected as one of the study drug since it is widely usedin the management of DM and the interaction studies with many of the concomitant therapies yet to be studied.

Based on the review of literature, it is clear that numerous herbal medicines, when taken in conjunction with antidiabetic pharmaceutical agents, could potentially alter their pharmacokinetic or pharmacodynamic Properties. Current study was conducted to evaluatethe pharmacological drug interaction of *H. rhamnoides*(400 mg/kg) with GLB (5 mg/kg) in diabetic rat model.

Weight loss is a major characteristic of Diabetes mellitus. Particularly, body weights of the disease control rats were lower than those treated with GLB, *H. rhamnoides* and combination of *H. rhamnoides* and GLB throughout the experimental period. The glycemic status of diabetic animals was reflected in blood glucose levels. Following 21 days treatment, GLB significantly ($P < 0.05$) reduced blood glucose levels compared to disease control group. As expected *H. rhamnoides* group showed significant ($P < 0.05$) reduction in the blood glucose levels compared to the disease control rats. When the allopathic drug and herbal drug were administered concomitantly, the hypoglycemic potential of the drug and herb improved. The combination group of *H. rhamnoides* and GLB was found most significant ($P < 0.05$) over the single treatment of *H. rhamnoides* and GLB in reducing blood glucose levels in STZ induced diabetes mellitus in wistar rats.

Diabetes is linked to hyperlipidemia. Inadequate control of blood glucose level may result in disturbance in the serum lipid profile. Reductions in serum lipid levels, particularly of the TG and LDL-C to normal levels are considered as beneficial for the long-term prognosis of diabetic patients.[19] The serum lipid levels observed in all treatment groups (GLB, *H.*

*rhamnoides* and *H. rhamnoides* + GLB), were found statistically significant ($P < 0.05$). This improvement might be due to the potent antioxidant effect of *H. rhamnoides* that makes it a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems.[20]

There are variety of *in vivo* antioxidant mechanisms that protect against the negative effects of free radical generation.[21] Diabetes mellitus has been linked to the changes in antioxidant defence systems.[22] As a result, antioxidant therapy may help to prevent and delay the onset of diabetes complications.[23] The presence of significant levels of vitamin C and E, carotenoids, phytosterol, tannins, and antioxidant enzymes in *H. rhamnoides* fruit contributes to its antioxidant activity.[24] In the present study, MDA levels were significantly ($P < 0.05$) decreased and Catalase levels were significantly ($P < 0.05$) increased in all treatment groups compared to those of disease control group and *H. rhamnoides* and GLB monotherapy. STZ produces partial to complete destruction of the pancreatic islets and vacuolation and necrosis of beta cells. In liver, STZ damages the hepatic parenchyma leading to necrosis of hepatocytes, reduction in sinusoidal spaces, etc.[25] All these changes were evident in disease control group during histopathological evaluation. All treatment groups (GLB, *H. rhamnoides* and *H. rhamnoides* + GLB) resulted in effective restoration of pancreatic islets and liver tissues. Therefore, combination of *H. rhamnoides* and GLB had a strong additive effect on maintaining beta cell and insulin production in STZ-induced diabetic rats.

## CONCLUSION

The present pre-clinical study was aimed to evaluate occurrence of *in-vivo* pharmacodynamic interaction wherein Glibenclamide and *Hippophae rhamnoides* were administered concomitantly. Based on the results obtained, all treatment groups, i.e., group 3 (GLB), group 4 (*H. rhamnoides*) and group 5 (*H. rhamnoides* + GLB), showed a significant ($P < 0.05$) increase in the weight of animals when compared with the disease control group. The combination treatment group revealed significant ($P < 0.05$) results over the single treatment of *H. rhamnoides* and Glibenclamide in the aspect of improving blood glucose levels and lipid profile. All treatment groups indicated a significant ($P < 0.05$) elevation in catalase levels and a significant ($P < 0.05$) reduction in MDA levels. Moreover, *H. rhamnoides* and GLB combination showed the greatest restoration effect on pancreatic and liver tissues during histopathological evaluation. Since combination therapy of *H. rhamnoides* and GLB had a synergistic effect on maintaining beta cell and insulin secretion in STZ-induced diabetic rats,

it can be concluded that the combination of the *H. rhamnoides* and GLB is more potent as compared to that of *H. rhamnoides* and GLB monotherapy.

A Pharmacokinetic Interaction study can be done on the same herb–drug combination in experimental rats. Further clinical research is required to examine results in diabetic patients by administering the same combination of antidiabetic drugs.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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