

# Hepatoprotective and antidiabetic effect of *Guaiacum officinale* in diabetes induced male albino wistar rats

Sadaf Ibrahim<sup>1</sup>, SNH Naqvi<sup>2</sup>, Rehana Perveen<sup>2</sup>, Hina Abrar<sup>3</sup>,  
Zuneera Akram<sup>2</sup>, Hina Tabassum<sup>3</sup> and Samina Sheikh<sup>1</sup>

<sup>1</sup>Department of Pharmacology, Faculty of Pharmacy, Zia Uddin Medical University, Karachi, Pakistan

<sup>2</sup>Department of Pharmacology, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan

<sup>3</sup>Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan

**Abstract:** The aim of present study is to evaluate the antidiabetic and hepatoprotective effect of *Guaiacum officinale* in streptozotocin induced male albino rats. The methanolic bark extract of *Guaiacum officinale* was administered at a dose of 500 mg/kg and Glibenclamide was used as a standard drug at a dose of 0.5mg/kg for 28 days. The animals were divided in to four groups. Control group n=12, received distilled water. Positive control group (STZ group) n=12 received streptozotocin at 30 mg/kg dose through I/P route. Standard group (STZ+ GLB group): n=12 received Glibenclamide. Treated group (STZ+ extracted group): n=12 received bark extract of *Guaiacum officinale*. Blood glucose level was significantly reduced after oral administration of bark extract in streptozotocin induced diabetic rats. The SGOT level significantly reduced in *Guaiacum officinale* treated group as compare to control, pronounced reduction of ALT level as compared to GLB and the ALP levels was highly significantly reduced in *Guaiacum officinale* treated group while GLB is unable to improve ALP levels in GLB treated diabetic albino rats. The level of direct bilirubin in *Guaiacum officinale* treated group was found to be insignificant as compared to control and STZ treated group while the level of indirect bilirubin was significantly reduced in STZ treated group as compare to control. Histopathological studies showed that *Guaiacum officinale* have hepatoprotective effect in experimental induced male albino rats.

**Keywords:** Diabetic, streptozotocin, hepatotoxic, glibenclamide, histopathological studies

## INTRODUCTION

Diabetes mellitus is a metabolic disorder and most common disease worldwide that causes the disturbance in metabolism of glucose, lipids and protein metabolism (Van den Berghe *et al.*, 2006) characterized by hyperglycemia, glucosuria, polyuria and weight loss. In Asia more than 99% population suffered from type-2 diabetes. The occurrence and consequences of diabetes type 2 in children are also increasing with its complications. Hyperglycemia is the most important parameter for the diagnosis of diabetes that results due to inability of pancreatic insulin secretion and its action or both. Hyperglycemia is the major reason that leads to serious damage of vital organs like liver, kidneys, heart, eyes and blood vessels. Chodury *et al.* (2002) reported that the evidence of micro and macro vascular diseases occur more in Asian patients as compared to Europeans. The International Diabetes Federation in 2012 reported that the people living with diabetes globally, that value will increase up to 565 million by 2030.

The plants belong to family *Zygophyllaceae* have antidiabetic properties (Satyavati *et al.*, 2007). *Guaiacum officinale* belongs to family *Zygophyllaceae*. The plant of family *Zygophyllaceae* useful for the treatment of piles, urinary disorders, dysentery, stomach disorder, cancer,

blood purifier and typhoid and for relief of constipation as laxative (Akhter *et al.*, 2009).

In the present study, the effect of *Guaiacum officinale* in streptozotocin induced diabetic rats and the protective effect of bark extract on liver. Liver is a vital organ that involved in absorption of nutrients from digestive tract, fat digestion and detoxification of toxins, drugs, medicines which causes the damages of vital organs (Krivoy *et al.*, 1996). Type 2 diabetic rat model in albino rats were prepared by the use of streptozotocin at 30 mg/kg dose by intraperitoneal route (Furman, 2015; Graff *et al.*, 2018). Scientists were used streptozotocin as diabetogenic agent but it also causes severe hepatotoxicity (Elizabeth *et al.*, 2017) nephrotoxicity as well as gastric ulceration.

## MATERIALS AND METHODS

### Identification of plant

The stem bark of *Guaiacum officinale* was collected from the trees grows in University of Karachi, Pakistan. It was identified by "Voucher specimen (No. 33 KU)" has been deposited in the Herbarium of Department of Botany, University of Karachi.

### Preparation of methanolic stem bark extract

The stem bark of *Guaiacum officinale* was dried in open air and shade, than chopped. The 10kg of air dried

\*Corresponding author: e-mail: hina.abrar@duhs.edu.pk

chopped bark of *Guaiacum officinale* was grounded and then extracted with methyl alcohol three times at room temperature. The methanol was get rid from the plant extract to produce a gummy residue (brownish) by freeze drying process under reduced pressure.

#### **Method for preparation of streptozotocin solution**

After overnight fasting (12-16 hours) Streptozotocin were used for induction of diabetes (sigma, St. Louis, MO). Streptozotocin injected by dissolving in freshly prepared "0.1 M sodium citrate buffer pH = 4.5." (Rajurkar, 2006), followed the metabolic changes occurred during pre-post streptozotocin injection for one week.

#### **Animal protocols**

A total (144) one hundred and forty four male albino wistar rats, weight 200±20 gm were selected for experimental study. Animals were procured from PCSIR (Pakistan Council of Scientific and Industrial Research) Laboratory of Karachi. The animals were kept in the animal house in an alternating 12 hours light/dark cycle at a temperature of 25±2°C for acclimatization of animal in Baqai Medical University. An approval has been taken from Institutional Ethics Committee of Baqai Medical University Karachi, before starting the experimental study. The animals were kept on same and uniform diet.

#### **Treatments group and dosing protocol**

144 male albino wistar rats were divided into four groups. Control group n=12, received distilled water. Positive control group (STZ group) n=12 received streptozotocin at 30 mg/kg dose through I/P route. Standard group (STZ+ GLB group): n=12 received Glibenclamide at 0.5 mg/kg dose by oral route for 28 days (Maurya *et al.*, 2012). Treated group (STZ+ extracted group): n=12 received bark extract of *Guaiacum officinale* at 500 mg/kg dose orally for 28 days. The experiment was repeated three times for evaluation of correct results.

#### **Biochemical assessment**

The blood samples were collected by cardiac puncture technique in vacutainer containing gel tubes (Feroz *et al.*, 2011). Blood was centrifuged by labofuge centrifuge machine, model 80-2S at a temperature 25°C at 3000 rpm (revolution per minute) for 15-20 minutes. After centrifugation serum (supernatant) was separated and used for liver enzymes (SGPT, SGOT, ALP and bilirubin evaluation). These enzymes were evaluated by standard reagents kit.

#### **Light microscopy**

After one month of treatment, the animals were sacrificed by cervical decapitation or cervical dislocation (Gandhi *et al.*, 2012). After dissection of animal organs immediately fixed in formalin solution for 24-48 hours. Dehydrated with ethyl alcohol, cleared with xylol solution, Infiltration of tissues carried out by melted paraplast at temperature of 58°C. Solid mass of paraplast consist of metal pieces of

L-shaped were used to fix the tissues. Cut 4-5 micron thick section, stained by Hematoxylin and Eosin (Bancroft 1990).

#### **STATISTICAL ANALYSIS**

Data entry and analysis carried out by SPSS version 20. Data were presented by Mean ± SEM. Level of significance \*p<0.05, \*\*p<0.01 and\*\*\* p<0.0001.

#### **RESULTS**

The effect of standard and treated drugs on blood glucose level described in table 1 (group 1) which showed that glibenclamid significantly (p<0.0001) improve the level of blood glucose as compared to streptozotocin induced diabetic group. While group 2 showed that *Guaiacum officinale* is significant (p<0.0001) improve the level of blood glucose in comparison of positive control group).

The effect of *guaiacum officinale* on liver functions in male albino rats showed in table 2. The elevation of liver enzymes seen due to hepatocellular damage in STZ induced diabetic group. The levels of ALT, AST and ALP enzymes significantly reduced by bark extract of *Guaiacum officinale* as compared to glibenclamide.

Table 3 showed the improvement of direct and indirect level of bilirubin by *Guaiacum officinale* as compared to positive control (STZ) group.

Histopathological studies of liver (fig 1 a,d,c,d,e,f) showed that streptozotocin significantly damage the cells of liver, fibrotic changes, congestion of central vein and lymphocytosis were observed by light microscopy as compared to control group. While the architecture of liver prominently improved by I in respect of hepatocellular damage, fibrotic changes, necrosis, parenchymal cells as compared glibenclamide group (showed in figs. 1g, h. i. j, k, l).

#### **DISCUSSION**

In worldwide the use of herbal medicine in traditional medical system has a positive impact for the treatment of various diseases especially on diabetes mellitus (Yu *et al.*, 2018). Diabetes mellitus are mostly developed due to overproduction of reactive oxygen species (Bonfont 2002). Most of the herbal medicine worked up by scavenging activity for reactive oxygen species (ROS) therefore it might be assumed that *Guaiacum officinale* worked up as an antioxidant. In the present study, the plant was to evaluate the hypoglycemic and hepatoprotective effects of *Guaiacum officinale* in streptozotocin induced diabetic male albino rats. The low dose of streptozotocin is beneficial for destruction of pancreatic β-cells by intraperitoneal route (Reed *et al.*,

2000) and the experimental evidence proved that they act by the free radicals formation and causes the intracellular change in DNA of pancreatic  $\beta$ -cells (Szkudelski 2001). Free radicals cause the damage of cellular molecules, proteins, DNA and lipids that leads to alter the cellular functions.

Hyperglycaemia is the key parameter for the diagnosis of diabetes that were induced by streptozotocin and it is associated with free radicals formations (Bajaj and Khan; 2012) that leads to increase peroxidation level, insulin resistance and inadequate antioxidant defence. The data of blood glucose of control group showed normal while the level was drastically increased in streptozotocin induced diabetic group as previously reported by researchers (Noor et al., 2008). In *Guaiaecum officinale* (treated group) the level was significantly reduced as compared to positive control group. When comparing the glucose levels in GLB treated group it was shown that the glucose was significantly reduced in GLB treated group as compared to positive control/STZ group (table 1). Similar observation was also reported by Shankar et al., 2001 and Tian et al., 1998. Thus the results described that STZ

treated group elevate the level of blood glucose by decreasing the level of insulin in plasma (Maiti et al., 2004). *Guaiaecum officinale* is as effective in reducing glucose level as GLB by increasing the effect of insulin in plasma, by potentiating the secretion of insulin from  $\beta$ -cells of islets of Langerhens or increase the glucose uptake by peripheral tissues. These results are also supported by antidiabetic effect of different herbal drugs (Lat Sas et al. 2014, Burcelain et al. 1995).

Liver function test are the major indicator of liver injuries and diseases. It is helpful for the identification of hepatic dysfunction, monitor the progression of disease and observe the effects of hepatotoxic drugs (Kim 2008). Hepatocellular damage increases the release of liver markers (AST, ALT, ALP and bilirubin). In the present study, the effect of *Guaiaecum officinale* at a dose of 500 mg/ kg by oral route for 28 days on liver function in streptozotocin induced diabetic rats was investigated. The elevation of liver enzymes (ALT, AST and ALP) reflects the hepatocytes injury in streptozotocin induced diabetic rat supported by Ramesh et al., 2007 (table 2). The level of ALT significantly reduced by *Guaiaecum officinale* as

**Table 1:** Comparison of blood glucose level between standard and treated group

Group 1	Blood Glucose level (Mean $\pm$ SEM)
Control	69.33 $\pm$ 1.281
Streptozotocin (positive control)	342.08 $\pm$ 10.546
Glibenclamide (standard group)	167.08 $\pm$ 15.287***
Group 2	Blood Glucose level (Mean $\pm$ SEM)
Control	75.000 $\pm$ 2.0412
Streptozotocin (positive control)	295.167 $\pm$ 10.9433
<i>Guaiaecum officinale</i> (Treated group)	133.667 $\pm$ 8.3814***

Data were presented by Mean $\pm$  SEM. Level of significance \*p<0.05, \*\*p<0.01 and \*\*\* p<0.0001

Group 1; Control, streptozotocin (positive control) standard group (glibenclamide)

Group 2; Control, streptozotocin (positive control), Treated (*Guaiaecum officinale*) group

**Table 2:** effect of *guaiaecum officinale* on liver functions in male albino rats

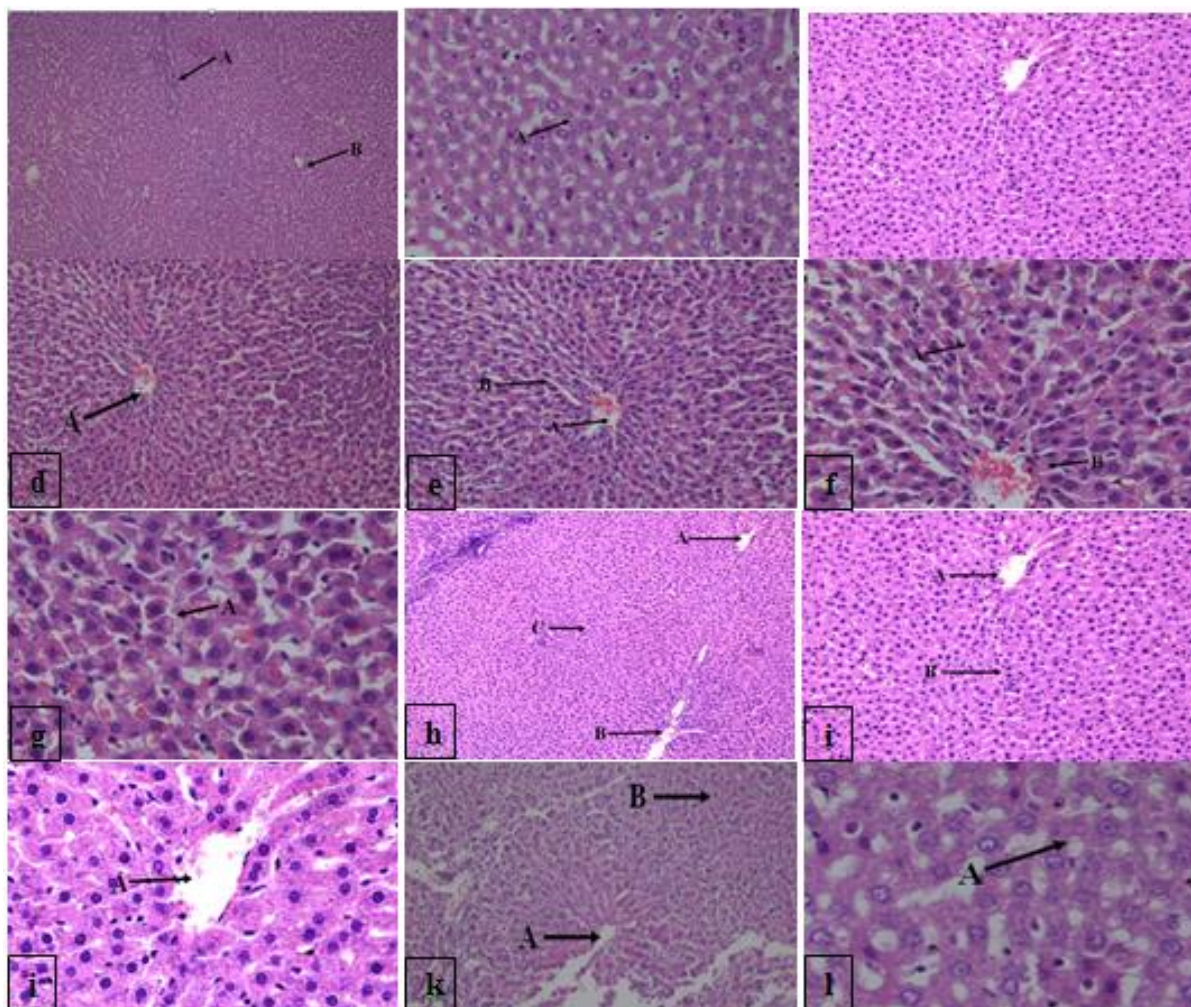
Groups	AST/SGOT	ALT /SGPT	Alkaline Phosphatase ALP
Control	216.09 $\pm$ 8.381	64.20 $\pm$ 2.471	177.50 $\pm$ 2.746
Diabetic group (Positive Control)	202.50 $\pm$ 5.084	80.00 $\pm$ 2.728***	581.50 $\pm$ 6.607***
Glibenclamide (Standard group)	215.80 $\pm$ 2.859	76.30 $\pm$ 4.531**	586.90 $\pm$ 2.953***
<i>Guaiaecum officinale</i> (treated group)	188.10 $\pm$ 7.391***	70.00 $\pm$ 1.390***	520.00 $\pm$ 2.485***

Data were presented by Mean $\pm$  SEM. Level of significance \*p<0.05, \*\*p<0.01 and\*\*\* p<0.0001.

**Table 3:** effect of *guaiaecum officinale* (500mg/kg) on direct and indirect bilirubin in male albino rats

Groups	Direct Bilirubin	Indirect Bilirubin
Control	0.055 $\pm$ 0.0054	0.112 $\pm$ 0.0063
Diabetic group (Positive Control)	0.044 $\pm$ 0.0047	0.068 $\pm$ 0.0051***
Glibenclamide (Standard group)	0.022 $\pm$ 0.0039***	0.090 $\pm$ 0.0059**
<i>Guaiaecum officinale</i> (Treated group)	0.052 $\pm$ 0.0049	0.119 $\pm$ 0.0038***

Data were presented by Mean $\pm$  SEM. Level of significance \*p<0.05, \*\*p<0.01 and\*\*\* p<0.0001.



**Fig. 1:** Photomicrograph of a 5 micron thick H&E stained paraffin section from the control male albino rat showing normal portal vein, normal portal tract and hepatocytes (a, b, c) X200 and X400. The section of positive control (STZ) showing mild and severe congestion in central vein, mild lymphocytosis and sinusoidal congestion X 200, X 400 (d, e, f). The section of standard group (Glibenclamide) group showing normal portal vein, Slight lobular inflammation, normal hepatocytes (g, h, i) X 200, X 400. The section from the treated (*Guaiacum officinale*) male albino rat showing normal central vein, normal hepatocytes and portal tract (j, k, l) X200, X 400.

compare to STZ induced diabetic group and AST level significantly reduced as compare to control. Researchers previously reported that the herbal drug belongs to family zygophyllacea was significantly reduces the level of AST and ALT in streptozotocin induced diabetic rats (Amin *et al.*, 2006). While the Glibenclamide did not able to reduce the level of AST as compared to diabetic group and the level of ALT slightly decreased by GLB as compare to diabetic group (table 2).

The level of ALP of positive control (STZ) group was highly significantly increased as compared to control. The level was highly significantly reduced in *Guaiacum officinale* (treated) group as compared to streptozotocin induced diabetic group while GLB is unable to reduce the level of ALP (table 2). The potential of hepatic damage

was observed when certain drugs like Alloxonor STZ were used to induce diabetes (Jasmine and Diasy, 2007). The above results of hepatic function depicted that STZ cause significant liver damage the study was also supported by previous research (El-Demerdash *et al.*, 2005). It was previously reported by several researchers that the herbal drug *Allium sativum* to decreases the level of ALT and AST in diabetic rats induced by streptozotocin as compared to Glibenclamide (Eidi *et al.*, 2006). *Tribulus terrestris* belongs to family Zygophyllaceae reduced the level of ALP in STZ induced diabetic rats (Maheswari *et al.*, 2015).

Bilirubin is a by-product of haemoglobin produced by liver called bile. The inefficient liver functions and fluctuation of albumin level associated with function of

hepatic cells (Muriel *et al.* 1992). In present study, determine the effect of *Guaiacum officinale* at 500mg/kg dose given orally on bilirubin was recorded. The results showed that *Guaiacum officinale* improve the levels of direct and indirect bilirubin as compared to positive control may be due to prevention of intracellular leakage of enzymes or by regeneration of cells supported by Thabrew *et al.*, 1992 (table 3).

Liver is a vital organ to maintain the normal glucose level and fluctuation in hepatic blood glucose causes the imbalance of auto oxidation and reduction reaction of hepatocytes. Liver or damage of any vital organ due to diabetes can be prevented by the selection of those drugs that must have antioxidant and blood glucose decreasing properties (Ramesh *et al.*, 2006 and Liu *et al.*, 2008). The histopathological studies of liver tissue of control group showed normal hepatic architecture, portal tract and hepatocytes during microscopic examination (figs. 1 a, b, c). The streptozotocin induced group (positive control) showed the congestion of central vein, mild lymphocytosis was seen. Sinusoidal congestion was seen. Any Fibrotic changes and necrosis were not seen in liver tissue of streptozotocin induced rats (fig. 1 d,e and f). These findings suggested that STZ cause considerable hepatic injury by oxidative stress and played a prime role in the development of diabetes complications (Kakkar *et al.*, 1995). These results were also supported by biochemical parameters. The histopathological observations of glibenclamide (standard) group of liver were found with normal hepatocytes. The congestion in the central vein and lobular inflammation were not seen. Parenchymal cell was almost appeared seen normal. Lymphocytes infiltration was not seen. Necrosis of tissues was not observed (figs. 1 g, h, i). The comparison of the histology of positive control with respect to standard group showed that GLB reduced the liver damage by managing of hyperglycaemia and oxidative stress (Kavishankar *et al.*, 2014). While the architecture of liver of *Guaiacum officinale* (treated) group was found that central vein was not dilated. Hepatocytes were seen normal. Necrotic and fibrotic changes were not seen. Portal tract and sinusoidal was seen normal. Parenchymal cell and lymphocytes were not seen in liver tissue of *G. officinale* treated group (figs. 1 j, k and l). The comparison of histology of standard and *Guaiacum officinale* treated group showed that both drugs are able to reduce the hepatic damage produced by STZ by inducing diabetes. In the light of above discussion it was concluded that *Guaiacum officinale* is an effective herb in reducing blood sugar level. Side by side this herb is very effective in reducing the major complication of diabetes mellitus like liver damage manifested as hepatocyte injury, biliary obstruction and oxidative stress induced by STZ (Ramesh *et al.*, 2006 and Efiog *et al.*, 2013).

## CONCLUSION

The present study was conducted to investigate the effect of herbal drug on hepatic functions, blood sugar level and histology of liver. The results provide valuable information pertaining hepatoprotective effects of *Guaiacum officinale* in STZ treated diabetic rats. However, the data of this present study needs to be more confirmed by using different advanced techniques like immunofluorescent or molecular studies.

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