

ANALYSIS OF ANTI-DIABETICS IN LIVER OF RATS

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Summary

In the present study deals with the anti-diabetic effect of *Gymnema sylvestre* (Retz) R.Br in liver of albino wistar rats. The histopathological and biochemical assays were carried out in organ and serum of liver. The different concentration of power treated as 5, 10, 15, 20/gms/25 days. The extent of diabetics was assessed in liver by measuring the levels of selected blood parameters of protein, glucose, cholesterol, insulin and triglycerides. The power reduced the liver weight of animals and reduced significant level of insulin, protein, triglycerides, cholesterol and glucose and affected the tissues.

Keywords: Anti-diabetic effect, *Gymnema sylvestre* (Retz) R.Br., Histopathology and Biochemistry in liver.

Introduction

Diabetes mellitus is a serious chronic metabolic disorder that has a significant impact on health, quality of life, and life expectancy of patients. In the United States, diabetes is the sixth leading cause of death (1)

Gymnema sylvestre, a plant native to the tropical forests of India has long been used as a treatment for diabetes. It is a traditional herb that helps to promote weight loss possibly through its ability to reduce cravings for sweets and control blood sugar levels. (200). When *Gymnema* leaf is placed directly on the tongue, it eliminates the sensation of sweetness, even if sugar is put in the mouth immediately. When taken internally, it helps to control blood-sugar levels in diabetes (2).

It has been used as a treatment of diabetes mellitus since ancient times. The leaves are used primarily to treat blood-sugar disorders, notably diabetes, stomachic, stimulant, purgative, laxative and diuretic (5, 6).

Liver is the chief organ involved in detoxifying the body. When the liver is over-stressed all other organs start to dysfunction. When the colon is overburdened with poor dietary consumption and accumulated toxicity, the liver must then work overtime in the attempts to purge the toxins and pollutants from the body. If the liver is unable to perform its full function, it will not only become even more toxic, but all the other organs are challenged in a domino effect. One of the liver's primary jobs is to store energy in the form of glycogen, which is made from a type of sugar called glucose. The liver removes glucose from the blood when blood glucose levels are high.

In the present study an attempt is made to explore the anti-diabetic activity in liver of rats using *Gymnema sylvestre* through the histopathological and biochemical activity of plasma insulin, glucose, triglycerides, protein and cholesterol.

Materials and Methods

Collection and Preparation of crude drugs

The plant was collected, identified and match with herbarium of Holy Cross College in Nagercoil, Kanyakumari District, Tamilnadu, India. The specimens were shade dried and powdered coarsely.

Experimental animals

Albino Wister rats of either sex, weighing 180-210g, were kept in separated cages under standard environmental conditions of temperature 20 to 30°C and humidity were provided with standard rat chow and water adlibitum. The experimental procedures were carried out in strict compliance with the institutional animal ethical committee regulations.

Treatment protocol

The animals were divided into 5 groups of 5 animals each and treated as per the protocol given below.

Control Group (G1): The animals were given normal diet of rice and milk for 25 days.

Treatment Group (G2): The animals were given 5g of powder with rice and milk.

Treatment Group (G3): The animals were given 10g of powder with rice and milk.

Treatment Group (G4): The animals were given 15g of powder with rice and milk.

Treatment Group (G5): The animals were given 20g of powder with rice and milk.

The reduction of body weight in rats was made to fast overnight after the experimental period. They were euthanized by anesthesia using chloroform vapour and blood was collected by cardiac artery bleeding and transferred into EDTA treated tube immediately. Blood was then centrifuged at 4000 rpm for 10 min to remove red blood cells and recover plasma. Liver were dissected out, weighed and preserved for histopathological studies. The liver from different groups was weighed and differences in weights were noted.

Histopathological studies

Liver sections were preserved in 10% formalin. They were stained with haematoxylin and eosin, the stained sections were observed under the microscope to estimate the extent of diabetic mellitus.

Biochemical tests

Estimation of glucose, protein, cholesterol, triglycerides and insulin were done using standard protocols.

Statistical analysis

All the values were expressed as Mean±S.D. The data was analyzed using mathematical calculations values and expressed as significant.

Results and Discussion**Biochemical parameters**

In the present investigation rats fed with *G. sylvestre* with different doses (5, 10, 15,20/g) showed a decrease in body weight, glucose level, cholesterol level, protein level, triglyceride level and insulin level when compared to the initial day at the end of the 25th day (Table 1.).

Table1. The Mean ± S.D of Body weight, plasma insulin, cholesterol, protein, triglyceride and glucose level of albino wister rat treated with *G. sylvestre*

Con. of dose (g/kg)	Mean ± S.D						
	Body Weight(gms)		Number of test in rats				
	Initial (wt)	Final (wt)	Cholesterol	Protein	Triglyceride	Insulin	Glucose
G1	173 ± 35.5	170 ± 35.0	87.25 ± 3.3	7.52 ± 0.4	55.80 ± 3.4	0.14 ± 0.02	90.43 ± 5.4
G2	180 ± 18.0	155.0 ± 2.60	72.02 ± 6.8	7.66 ± 0.3	46.59 ± 4.8	0.22 ± 0.05	89.51 ± 4.6
G3	126± 23.6	104.2 ± 22.4	89.30 ± 4.7	7.80 ± 0.4	43.89 ± 5.6	0.14 ± 0.02	88.16 ± 5.0
G4	199 ± 19.8	145.6 ± 36.2	51.44 ± 6.7	8.04 ± 0.5	38.64 ± 3.2	0.07 ± 0.01	85.03 ± 7.2
G5	176 ± 36.5	131.4 ± 22.0	86.31 ± 3.7	8.14 ± 0.5	35.66 ± 2.8	0.04 ± 0.01	53.66 ± 4.6

Histopathological studies:

The results of histopathological studies are shown in Plate 1.

Treatment G1 (0g) - The sections from G1 group showed normal hepatocytes, normal liver parenchyma (Control).

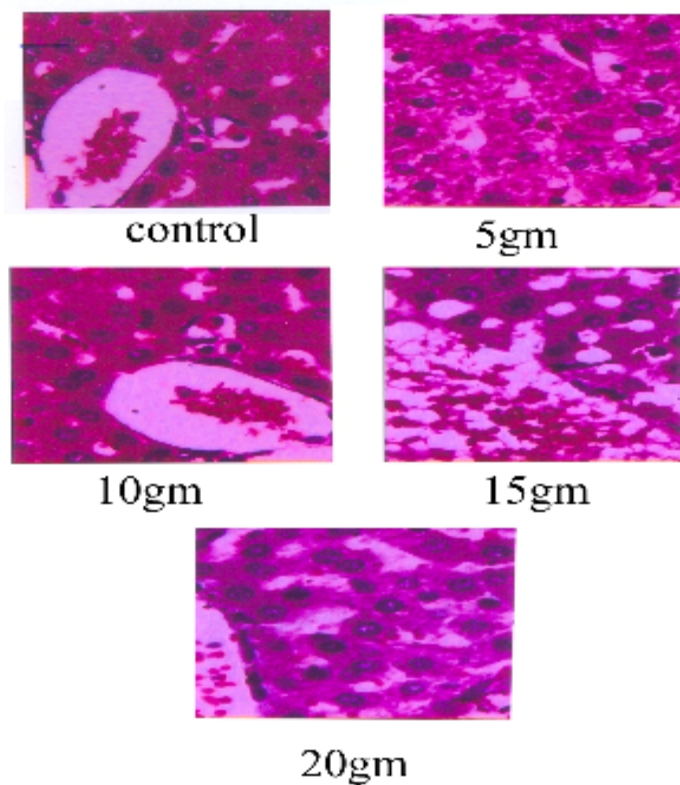
Treatment G2 (5g) - The section treated with the 5gm plant powder showed liver parenchyma with sheets of hepatocytes showing slightly changes and areas of necrosis.

Treatment G3 (10g) - The sections from powder treated group showed liver parenchyma with sheets of hepatocytes showing hydropic change and areas of early necrosis.

Treatment G4 (15g) - The sections from powder treated group showed liver parenchyma with sheets of hepatocytes showing hydrophic change and mild fatty change, areas of mild necrosis were seen sinusoids showed dilation.

Treatment G5 (20g) - The sections from powder treated group showed liver parenchyma with sheets of hepatocytes showing hydropic change, fatty change and areas of severe necrosis. There was dense periportal inflammatory infiltrate and periportal inflammatory infiltrate and periportal diabetes mellitus, the picture was suggestive of severe hepatic injury and early diabetes mellitus.

Plate.1- The histopathological effect on liver rats



Liver helps clear toxic substances such as drugs from the blood stream by absorbing the harmful substances, chemically altering them and then excreting them in the bile. In the present study high dose group G4 and G5 treated with *G. sylvestre* causes hepatic changes where as lower doses does not alter the normal histology.

The protective effect of *Annona squamosa* on the liver of normal and diabetic rabbits was studied. Similar changes were observed in the liver in which, the hepatocytes exhibited severe ballooning degeneration with early steatohepatitis in some foci. In addition, massive hepatocyte necrosis with mallory body formation and extensive haemorrhage was also evident. Extract of *S. longepedunculata* significantly increased the levels of serum AST and ALT (7).

The body weight gain and food intake decreased in a dose dependent manner in the rats administered with *Gymnema sylvestre*. The decrease in body weight gain may have been due to the decrease in food intake. Moreover *Gymnema* is known to suppress the receptor of taste of sweet on the tongue may be another cause. The decrease in body weight may be because of the low feed intake and alteration in the cholesterol, triglycerides and glucose levels in the serum (3).

The experimental plant leaves raise insulin levels, according to research in healthy volunteers (9), possibly due to regeneration of β -cells of the pancreas (10).

Effect of *Gymnema sylvestre* on the blood glucose level of normal male albino rats fed on different concentration of the herbal powder for a period of 25 days reveal a positive correlation between the drug and the glucose level (8). In *in vivo* studies have indicated that extracts of this power containing gymnemic acid suppress the elevation of blood glucose levels by inhibiting glucose uptake in the intestine.

However, in rats receiving a normal diet, no significant suppression of weight gain was observed (11).

The experimental plant leaf extract: a 52-week dietary toxicity study in wistar rats (12). The histopathological studies were carried out in liver of rats. The liver fibrosis was induced by ccl_4 and extent of disease was assessed by measuring the level of HP and TBL using *Trigonella foenum-graecum* (13).

When the glucose level is already less due to this plant leaves the need for insulin is reduced. This could be the reason for the decreased level of insulin. In the G1 group animals and at low dose group of G2 and G3 the insulin levels were found to be normal.

Reduction in plasma cholesterol, triglycerides, and free fatty acid levels was observed in two studies of diabetic patients who received supplements of these leaves in addition to their usual antidiabetic medication (eg, insulin, glibenclamide or tolbutamide) In contrast; these levels increased gradually from baseline in the control group patients not taking these dose. It should be noted that lipid lowering was a secondary endpoint in these studies, which were designed to demonstrate the antidiabetic effects of *Gymnema*.

Conclusion

G. sylvestre does not pose severe threat to the diabetics at concentration up to 10 gm/kg body weight. Higher concentrations of 15 and 20 gm/kg body weight though causes histopathological change the severity is very much reduced and the dosage of up to 10gm/kg body weight does not affect the protein, glucose, insulin, cholesterol or triglycerides Thus it could be recommended that *G. sylvestre* could be used in various ailments in limited dosage on the advice of a physician.

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