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Anti-Diabetic Effect of *Gymnema sylvestre* (Asclepiadaceae) Powder in the Stomach of Rats

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ABSTRACT

The present study reveals the anti-diabetic effect in stomach of albino wistar rats using ***Gymnema sylvestre* (Retz) R.Br.** herbal powder. The histopatholoical and biochemical assays were carried out in organs and blood serum of stomach. The different concentration of ***G. sylvestre*** treated as 5, 10, 15, 20/gms/25 days. The effect of crude drugs in rats reduced the body weight and the diabetics was assessed in stomach by measuring the levels of selected blood parameters of protein, glucose, cholesterol, insulin and triglycerides and the effect of histopathology. Treatment with ***G. sylvestre*** reduced the stomach weight of animals and reduced significant level of insulin, protein, triglycerides, cholesterol and glucose. The data was analyzed using mathematical calculation values and were expressed as significant.

Keywords: Anti-diabetic effect**,** ***Gymnema sylvestre* (Retz) R.Br.,** Histopathological and Biochemical assay in stomach.

INTRODUCTION

Gymnema sylvestre (Asclepiadaceae) is a large tropical liana native to central and western India and can be also found in tropical Africa and in Australia **(Stocklin, 1969).** The plant is stomachic, stimulant, laxative and diuretic. It is good for cough, biliousness and sore eyes. If the leaves of the plant are chewed, the sense of taste for sweet and bitter substances is suppressed. The leaves are said to be used as a remedy for diabetes **(Grover *et al.*, 2002; Gholap** and **Kar, 2003).** It has shown effective activity against ***Bacillus pumilis, B.subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Satdive *et al*., 2003).**

The users of these health foods often expect weight reduction or improvement of diabetes because of their ability to suppress the taste of sweetness and inhibit glucose absorption **(Nakamura 1988, Ueno 1997).** The constituents which effectively work on diabetes in ***G. sylvestre*** leaves are gymnemic acids **(Murakami *et al*., 1996).**

Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities and altered intermediary metabolism of major food substrates. **(Scoppola *et al*., 2001).**

Cases of rare hepatic inflammation possibly induced by anthraquinone derivatives have also been reported **(Nadir, 2000)** and may be dose related. It was suggested that the anthraquinones may be metabolized in the intestine to form a hepatotoxic compound that some people were sensitive to, which ultimately resulted in reversible liver damage.

Gastrointestinal complaints rank among the most frequent reasons why people ask for medical advice. About 15-30% of the adult patients suffer from different various functional dyspeptic conditions. The therapy of functional gastrointestinal disorders is one of the domains of phytotherapeutic treatments. From ancient times on, bitter herbal drugs played a very important role in the therapy of patients with dyspeptic symptoms.

The mechanisms of action of the bitters are not completely understood. But there are indications that they sensorially stimulate at even very small concentrations the secretion of the stomach as well as the digestive glands and strengthened digestive tract seems to stimulate the central nervous system, leading to a general tonification. At higher dosages bitters probably directly affect the mucous membranes of the stomach and the bowel. Bitters often are combined with essential oils (some volatile oils as aromatic bitters, drug combinations of a volatile oil with a bitter). Essential oils act primarily as spasmolytics, carminatives and local anesthetics (**Saller *et al*., 2001).**

In the present study, the effects of different concentrations of **Gymnema sylvestre** in the albino wistar rats through histopathological and biochemical reaction in rats.

MATERIALS AND METHODS

Collection and Preparation of crude drugs:

The plant was collected and identified in the herbarium of Holy Cross College in Nagercoil 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 30ºC and humidity were provided with standard rat chow and water *ad libitum.* The experimental procedures were carried out in strict compliance with the institutional animal ethical committee regulations.

Study protocol:

A pilot study was conducted to study the dose response relationship of the plant powder of ***Gymnema***, a dose of 5, 10, 15, 20, 25 gms with rice and milk for a period of 25 days.

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 ***Gymnema*** powder with rice and milk.
Treatment Group (G3): The animals were given 10g of ***Gymnema*** powder with rice and milk.
Treatment Group (G4): The animals were given 15g of ***Gymnema*** powder with rice and milk.
Treatment Group (G5): The animals were given 20g of ***Gymnema*** 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. Stomach were dissected out, weighed and preserved for histopathological studies. The stomach from different groups was weighed and differences in weights were noted.

Histopatholical studies:

Stomach 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: The reagent mixture (Phosphate buffer pH 7.5:250 mmol/1, Phenol 5 mmol/1, 4-Aminoantipyrin 0.5 mmol/1, Glucose oxidase (GOD) = 10 KU/1, Peroxidase (POD), =1 KU/1) was taken in three

test tubes each 1000µl standard (S), test (T) and blank (B).10µl of serum was added to (T). Standard 10µl/100mg/dl solution was added to (S) and 10 µl of distilled water was added to (B). The contents were mixed well and incubated for 10min at 37°C. The absorbance of (S) and (T) was read against reagent (B) spectrophotometrically. The intensity was measured at 505nm.

***Estimation of protein**:* The Biuret reagent mixture Sodium hydroxide 500 mmol/1, Potassium sodium tartrate 35 mmol/1, Potassium iodide 30 mmol/1, Copper sulphate10 mmol/1) was taken in three test tubes each 1000µl standard (S) and test (T) blank (B) and 10µl of serum was added (T). Standard 10µl/6 g/dl solution was added to (S) and 10 µl of distilled water was added to (B). The contents were mixed well and incubated for 10min at 37°C. The absorbance of (S) and (T) was read against reagent (B) spectrophotometrically. The intensity was measured at 546nm.

Estimation of cholesterol: The reagents mixture (Good's buffer pH 6.7:50 mmol/1, Phenol5 mmol/1, 4-Aminoantipyrine 0.3 mmol/1, Cholesterol eaterase (CHE) = 200 U/1,
Cholesterol oxidase (CHOD) = 50 U/1, Peroxidase (POD) = 3 kU/1) were taken in three test tubes each 1000µl standard (S) and test (T) blank (B) and 10µl of serum was added (T). Standard 10µl/200mg/dl solution was added to (S) and 10 µl of distilled water was added to (B). The contents were mixed well and incubated for 10min at 37°C. The absorbance of (S) and (T) was read against reagent (B) spectrophotometrically. The intensity was measured at 505nm.

***Estimation of triglycerides**:* The reagents mixure Good's buffer pH 7.2:50 mmol/1, 4-Chlorophenol 4 mmol/1, ATP 2 mmol/1, Mg^{2+}15 mmol/1,Glycerokinase (GK) = 0.4 kU/1, Peroxide(POD) = 2 kU/1, Lipoprotein lipase (LPL)=2kU/1, 4-Aminoantipyrine0.5 mmol/1, Glycerol-3-phosphate-oxidase= 0.5 kU/1)) were taken in three test tubes each 1000µl standard (S) and test (T) blank (B) and 10µl of serum was added (T). Standard 10µl/200mg/dl solution was added to (S) and 10 µl of distilled water was added to (B). The contents were mixed well and incubated for 10min at 37°C. The absorbance of (S) and (T) was read against reagent (B) spectrophotometrically. The intensity was measured at 546nm.

Estimation of insulin: The quantitative measurement of insulin was done by solid phase competitive enzyme linked immunosorbent assay (ELISA). Insulin was estimated in Vivek laboratories, Nagercoil.

Statistical analysis:

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

RESULTS AND DISCUSSION:

Biochemical parameters:

In the present investigation rats fed with ***G. sylvestre*** to G2 showed a decrease in body weight from 179.0 ± 17.8 to 159 ± 12.4 gm. Similarly in animals fed with powder to G3, G4 and G5 showed significant decrease when compared to the initial day at the end of the 25th day (Table 1.).

In G2 there was a slight increase (0.21 ± 0.05) when compared to the G1 (0.14±0.02). But G4 and G5 showed a decrease in insulin level when compared to the G1 ***G. sylvestre*** did significantly affect the plasma insulin profile of normal male albino rats at higher concentration group of G4 and G5 respectively. (Table 2.).

The glucose level showed a normal value of 89.40 ± 5.4, 87.56 ± 3.8 and 84.48 ± 4.4 in the G1, G2 and G3 respectively. However, at higher concentrations of G4 and G5 the glucose content decreased steadily with maximum decrease at G5. Higher concentration groups of G4 and G5 the glucose content decreased steadily with maximum decrease at G5 (Table 3.).

The G2 and G3 failed to produce statistically significant reduction. But G4 and G5 7.66 ± 0.2 and 7.80 ±

0.1mg/dl) showed a slight increase when compared to the G1 (7.52 ± 0.3 gm/dl). ***G. sylvestre*** does not significantly affect the plasma protein profile of normal male albino rats even up to higher concentration groups of G5 (Table 4.).

The triglyceride level showed a normal value of 90.43 ± 5.4, 89.39 ± 4.44 and 85.45 ± 5.5gm/dl in the G1, G2 and G3 respectively. However, at higher concentrations of G4 and G5 the value decreased steadily with maximum decrease at G5 (Table 5.).

The cholesterol level of 72.02 ± 6.8 and 51.44 ± 6.7 mg/dl was noted in the rats fed with ***Gymnema*** to G4 and G5 when compared to the control value of 89.3 ± 4.7mg/dl. The low dose group of 5 and 10 gm was similar to normal (Table 6.).

Table1. Body weight of albino wister rats treated with ***G. sylvestre*.**

Treatment groups	Mean Body Weight ± S.D (gms)		
	Initial Weight	Final Weight	Percent change
G2	179.0 ± 17.8	157.0 ± 12.4	-22
G3	126.4 ± 23.5	103.8 ± 22.2	-22
G4	198.0 ± 19.8	145.4 ± 36.1	- 52
G5	175.0 ± 36.3	130.9 ± 21.9	- 44

Table 2. Plasma insulin level of albino wister rat treated with ***G. sylvestre*.**

Treatment groups	Insulin µI/ml					
	Number of rats					Mean ± S.D
	1	2	3	4	5	
G1	0.13	0.12	0.14	0.18	0.13	0.14±0.02
G2	0.14	0.21	0.30	0.20	0.24	0.21 ± 0.05
G3	0.15	0.16	0.12	0.10	0.16	0.13 ± 0.02
G4	0.06	0.06	0.07	0.07	0.08	0.06 ± 0.01
G5	0.02	0.02	0.04	0.04	0.05	0.03 ± 0.01

Table 3. Plasma glucose level of albino wister rat treated with ***G. sylvestre*.**

	Concentration of glucose

Treatment groups	(mg/dl)					
	Number of rats					Mean ± S.D
	1	2	3	4	5	
G1	80.41	94.69	91.13	89.70	91.70	89.40 ± 5.4
G2	83.41	86.13	93.27	84.27	90.74	87.56 ± 3.8
G3	79.20	79.00	89.39	87.12	87.71	84.48 ± 4.4
G4	73.14	76.13	68.95	77.21	77.45	85.03± 3.2
G5	47.55	51.75	56.30	57.30	48.96	53.66 ± 3.8

Table 4. Plasma protein level of albino wister rat treated with ***G. sylvestre*.**

Treatment groups	Concentration of protein (mg/dl)					
	No: of rats					Mean ± S.D
	1	2	3	4	5	
G1	7.6	7.5	6.9	7.9	7.5	7.52 ± 0.3
G2	8.1	7.6	7.5	7.5	7.2	7.66 ± 0.2
G3	8.1	8.4	8.1	8.1	8.3	7.80 ± 0.1
G4	8.7	7.9	8.5	7.8	8.2	8.04 ± 0.3
G5	8.5	8.3	8.3	8.3	8.5	8.14 ± 0.1

Table 5. Plasma triglyceride level of albino wister rat treated with ***G. sylvestre*.**

Treatment groups	Concentration of triglyceride (mg/dl)					
	Number of rats					Mean ± S.D
	1	2	3	4	5	
G1	52.32	58.69	48.78	54.85	48.70	55.80 ± 3.7
G2	42.32	45.13	43.73	44.74	50.65	46.59 ± 2.8
G3	39.10	41.39	39.85	47.95	47.53	43.89 ± 3.8
G4	38.18	36.72	38.91	37.29	37.49	38.64 ± 0.7
	33.40	35.75	35.35	34.80	32.78	35.66 ± 1.1

G5						

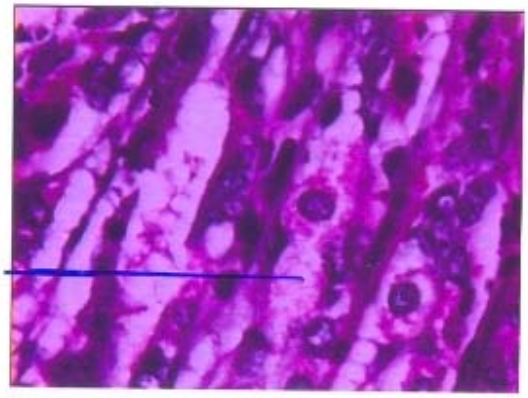
Table 6. Plasma cholesterol level of albino wister rat treated with ***G. sylvestre*.**

Treatment groups	Concentration of cholesterol (mg/dl)					
	Number of rats					Mean ± S.D
	1	2	3	4	5	
G1	91.40	80.52	87.39	89.11	91.30	0.14± 4.0
G2	85.74	83.59	83.25	84.37	90.78	0.21 ± 2.7
G3	79.15	87.29	87.31	87.31	87.38	0.13 ± 3.2
G4	61.01	69.21	75.29	76.51	77.56	0.06 ± 6.1
G5	44.39	43.23	57.35	48.78	48.98	0.03 ± 4.9

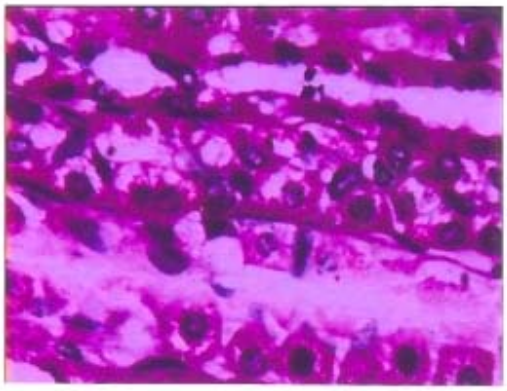
Histopathological Studies

The results of histopathological studies are shown in Plate 1.

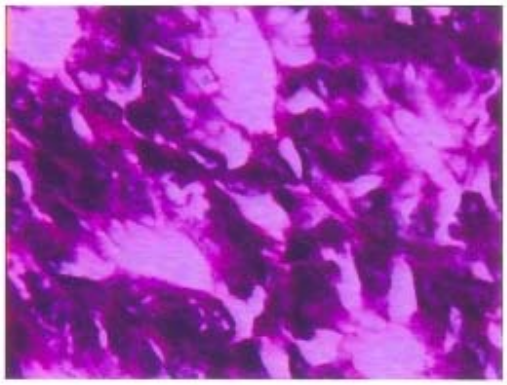
Plate 1. Histopathology in stomach of albino wister rats.



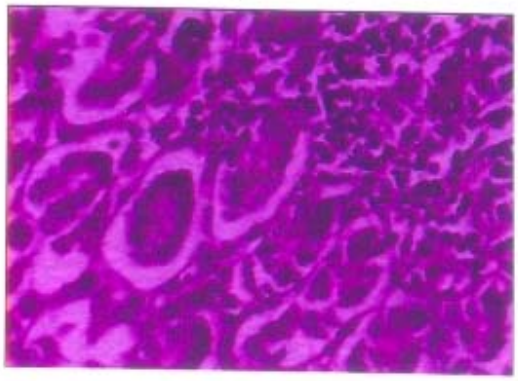
control



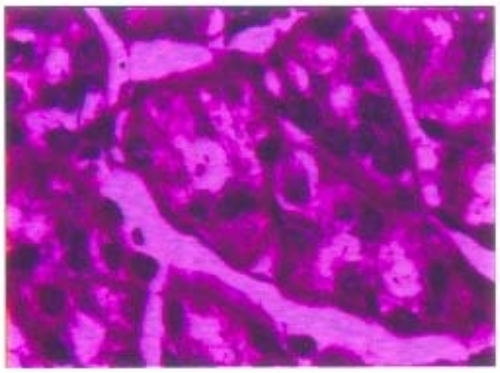
5gm



10gm



15gm



20gm

The sections from G1 showed normal histology of the stomach. The wall of the stomach consists of four layers: a mucosa, a submucosa, a muscularis externa, and a serosa. The mucosa is in the innermost layer, and reveals three distinctive regions. The most superficial regions contain the gastric pits; the middle region contains the necks of the glands, which tends to stain with eosin; and deepest part of the mucosa stains blue. The inner surface of the empty stomach is thrown into long folds referred to as rugae. It consists not only of mucosa but also of sub mucosa. The rugae are not permanent folds, and they disappear when the stomach mucosa is stretched, as in the distended stomach. The luminal surface of the stomach is pitted with numerous tiny openings, called gastric pits. These are formed by the luminal epithelium that invaginates the underlying connective tissue, lamina propria of the mucosa. The tubular gastric glands are located below the luminal epithelium, and they open directly into the gastric pits to

deliver their secretions into the stomach lumen. The gastric glands descend through the lamina propria to the muscularis mucosae. The stratified squamous eptithelial cells, gastric pits, submucosyl layer, muscosyl cells are clearly observed. No marked changes are observed in G2, G3 and G4. Animals fed with ***Gymnema*** to G5 showed a shrinkage of stomach which is clearly observed. No clear ulceration of stomach is visible.

According to Organization for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD_{50} dose of 2000 mg/kg of ***G. sylvestre*** and above is categorized as unclassified and hence the drug is found to be safe. In our present study dose up to G5 body weight did not show significant mortality but behavioural changes were observed. G2 and G3 of ***G. sylvestre*** were well tolerated by the animals without any behavioral changes during long-term treatment, but G4 and G5 showed lethargic movements and suppression of appetite.

The body weight gain and food intake decreased in a dose dependent manner in the rat's administred with ***G. 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. **Marquet *et, al.,* (1997)** considered that the amphiphilic characteristic of the molecules were responsible for their deleterious effects on the digestive tract.

Dose dependent changes were also observed by **Murlidara 1999** in fenugreek seeds. In the present observation no significant mortality was observed. The possible reason could be that ***Gymnema*** does not interfere with the various vital functions of the animal and thus not posing severe toxicity that may lead to the death of the animal **(Shanmugasundaram *et al.,* 1983).** The dose dependent changes both on feed intake as well as body weight. The reasons for the reduced feed intake could be the faint aromatic odour, tastelessness i,e. the sense of taste for sweet and bitter substance is suppressed **(Deepak 2006).**

The decrease in insulin level at higher concentration of G5 to reduced the glucose content in the serum there by reducing the secretion of insulin. Since it is a hypoglycemic drug, higher concentrations would reduce the glucose level of blood. The main function of insulin is to reduce the glucose level in blood. When the glucose level is already less due to ***Gymnema*** the need for insulin is reduced. This could be the reason for the decreased level of insulin. In the control animals and at low dose of G2 and G3 of ***Gymnema*** the insulin levels were found to be normal. According to **Ogawa et al., 2004** ***Gymnema*** are considered to be insulinotrophic and promote insulin secretion.

Studies on the changes in the level of plasma proteins on the animals fed with different concentrations of ***G. sylvestre*** showed no significant changes and all the doses were similar to the control. Thus the herbal powder does not have any effect on the protein level in rat. The possible reason for the decrease could be that the animals are normal and not exhibiting hyperglycemic condition or hypercholestremic condition.

There are no significant changes were observed in the levels of serum cholesterol and triglyceride levels in animals fed with low dose of ***Gymnema sylvestre*** which may be due to the normal diet and normal rats as observed by **Yumiko *et al.,* 1999.** In the higher concentration of G4 and G5 there was a decrease in cholesterol which may be because the gymnemagenin and gymnemic acids interacted with steroids, especially cholesterol and CA-derived bile acids in the intestinal tracts of rats.

Reduction in plasma cholesterol, triglycerides, and free fatty acid levels was observed in 2 studies of diabetic patients who received supplements of ***Gymnema*** in addition to their usual antidiabetic medication (eg, insulin, glibenclamide, or tolbutamide**) (Baskaran *et al.,* 1990).** In contrast, these levels increased gradually from baseline in the control group patients not taking ***Gymnema.*** It should be noted that lipid lowering was a secondary endpoint in these studies, which were designed to demonstrate the antidiabetic effects of ***Gymnema.***

G. sylvestre caused varying effects on guinea pig and rat ileal longitudinal muscles in the inverted intestine of the animals. It is suggested that Gymnemic acid suppress the glucose levels by inhibiting glucose uptake in the intestine.

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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