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The Effect of Anti-Diabetics in Kidney of Rats

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Abstract

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

Keywords: Anti-diabetic effect**,** *Gymnema sylvestre*, Histopathological and Biochemical assay in Kidney.

Introduction

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbal Medicines are readily available in the market from health food stores without prescriptions and are widely used in India, China, USA and all over the world. According to recent survey the majority of people who use herbal medicines do not inform their physicians about their consumptions that can cause abnormal test results and confusion in proper diagnosis. Drug herb interactions can result in unexpected concentration of therapeutic drug. Several herbal products interfere with immunoassays used for monitoring the concentrations of therapeutic drugs. Herbal medicines can also cause undesired effects. Therefore, the common belief that anything natural is safe is not correct.

Gymnema sylvestre is a large woody climber. Its principle constituent is gymnemic acid which has anti-diabetic and hyperglycemia properties. It abolishes the taste of sugar and is believed to neutralize excessive sugar present in the body in diabetes mellitus[1].

Whole 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 gymenic acid is made up of molecules whose atom arrangement is similar to that of glucose molecules. Those molecules fill the receptor locations on the taste buds for a period of one to two hours, thereby preventing the taste buds from being activated by any sugar molecules present in the food. Similarly the glucose-like molecules in

the gymnemic acid fill the receptor locations in the absorptive external layers of the intestine thereby preventing the intestine from absorbing the sugar molecules.

Unarguably, two of the mostly overlooked when it comes to detoxification are the liver and kidneys. When the liver is overworked the kidneys try to pick up the slack. Kidneys are two bean-shaped organs, each about the size of your first. Although the kidneys are small organs by weight, they receive a huge amount (approximately 20%) of the blood pumped by the heart. Every day, the kidneys process about 200 quarts of blood, to sift out 2 quarts of waste products and excess water. Without the kidneys, waste products and toxins would build up to dangerous levels in the blood and subsequently damage the body. Consequently through toxic-buildup or dietary neglect these important toxin-filtering organs can become inflamed, infected, and develop kidney stones or fail altogether.

As with virtually all the organs of the body, the kidneys are subject to a range of disorders and diseases. Kidney damage usually takes place gradually and without symptoms and it may only be after years of kidney disease that kidney functioning becomes noticeably decreased. In kidney disease one can generally lose more up to 75% of kidney functions before the effects become acutely obvious. If left untreated, kidney disease can become irreversible and can be a significant threat to your health. People with diabetes, high blood pressure or a family history of kidney disease also need to actively prevent kidney disease and keep the organs in optimum condition.

Kidney disease associated with aristolochic acid (called Chinese Herbal Nephropathy) can lead to end-stage kidney failure relatively quickly. Chinese Herb nephropathy was first identified in Belgium in the 1990's. About 100 patients with kidney failure were found to have taken a "slimming regimen" that was later identified as aristolochic acid. The kidney biopsies among these patients were very similar showing extensive and severe scarring of the kidneys. At least 70 of those patients went on to require dialysis or kidney transplantation. Additional cases of Chinese Herbal nephropathy were later identified in France, United Kingdom and the United States. As a result, in 2001, the U.S. FDA issued a severe warning and import ban for dietary supplements containing this compound.

In the present study, the effects of different concentrations of *G. 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 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 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 animas 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. Kidney were dissected out, weighed and preserved for histopathological studies. The Kidney 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^{o}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 standard (S) and 10 µl of distilled water was added to (B). The contents were mixed well and incubated for 10min at 37^{o}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^{o}C. The absorbance of (S) and (T) was read against reagent (B) spectrophotometrically. The intensity was measured at 505nm.

Estimation of triglycerides*:* The reagents mixture 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^{o}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 178.0 ± 17.6 to 156 ± 12.2 gm. Similarly in animals fed to G3, G4 and G5 showed significant decrease when compared to the initial day at the end of the 25th day (Table 1.).

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

Treatment groups	Mean Body Weight ± S.D (gms)		
	Initial Weight	Final Weight	% of value
G2	178.0 ± 17.6	156.0 ± 12.2	-22
G3	126.1 ± 23.4	101.6 ± 22.1	-22
G4	197 ± 19.6	144.3 ± 35.8	- 54
G5	174 ± 36.2	130.5 ± 21.7	- 45

In G2 there was a slight increase (0.20 ± 0.05) when compared to the G1 (0.13 ± 0.02). But G4 andG5 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 groups of G4 and G5 respectively (Table 2.).

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

Treatment groups	Insulin µI/ml					
	Number of rats					
	1	2	3	4	5	Mean ± S.D

G1	0.12	0.11	0.13	0.17	0.12	0.13 ± 0.02
G2	0.13	0.19	0.29	0.19	0.23	0.20 ± 0.05
G3	0.14	0.15	0.11	0.09	0.15	0.29 ± 0.02
G4	0.05	0.03	0.06	0.05	0.07	0.05 ± 0.01
G5	0.01	0.01	0.03	0.03	0.04	0.02 ± 0.01

The glucose level showed a normal value of 88.98 ± 4.6, 84.87 ± 4.3 and 83.70 ± 4.2 in the G1, G2 and G3 respectively. However, at higher concentrations group 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.).

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

Treatment groups	Concentration of glucose (mg/dl)					
	Number of rats					Mean ± S.D
	1	2	3	4	5	
G1	80.39	93.98	90.98	88.90	90.80	88.98 ± 4.6
G2	82.98	85.73	92.75	83.28	79.64	84.87 ± 4.3
G3	78.75	78.50	88.74	86.13	86.38	83.70 ± 4.2
G4	72.83	75.68	67.75	78.11	76.75	74.22 ±3.6
G5	46.99	50.85	55.93	55.93	47.98	51.64 ± 3.1

The G2 and G3 failed to produce statistically significant reduction. But G4 and G5 (7.94 ± 0.4 and 8.12 ± 0.1mg/ dl) showed a slight increase when compared to the G1 (7.16± 0.3 gm/dl). G. sylvestre does not significantly affect the plasma protein profile of normal male albino rats even up to a higher concentration of G5 (Table 4.).

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	7.2	7.3	6.5	7.5	7.3	7.16± 0.3
G2	7.9	7.1	7.3	7.3	6.9	7.30 ± 0.3
G3	7.8	8.2	7.8	7.8	8.1	7.94± 0.1
G4	8.5	7.5	8.3	7.5	7.9	7.94 ± 0.4
G5	8.3	8.1	8.1	7.9	8.2	8.12 ± 0.1

The triglyceride level showed a normal value of 51.47 ± 3.8, 44.75 ± 2.7 and 42.30± 3.7 gm/dl in the G1, G2, and G3 respectively. However, at higher concentration groups of G4 and G5 the value decreased steadily with maximum decrease at G5 (Table 5.).

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	51.38	57.78	47.18	53.18	47.85	51.47 ± 3.8
G2	42.86	45.38	42.38	43.38	49.78	44.75 ± 2.7
G3	38.14	41.76	38.45	46.53	46.65	42.30± 3.7
G4	37.45	35.87	37.92	36.83	36.73	36.96 ± 0.6
G5	33.48	34.58	34.58	33.70	31.45	33.55 ± 1.1

The cholesterol level difference of 72.05 ± 4.0 and 49.40 ± 5.9 mg/dl was noted in the rats fed with *Gymnema* to G4 and G5 compared to the G1 value of 87.15 ± 3.6 mg/dl. The low dose group of G2 and G3 was similar to normal (Table 6.).

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	90.80	79.98	86.28	88.10	90.60	87.15 ± 3.6
G2	85.34	82.52	82.24	83.54	79.38	82.60 ± 1.9
G3	78.85	86.54	86.39	86.38	86.30	84.89 ± 3.0
G4	67.03	68.38	74.39	76.45	76.38	72.05 ± 4.0
G5	43.89	42.45	56.34	56.38	47.96	49.40 ± 5.9

Histopathological studies

The results of histopathological studies are shown in Plate 1.

The sections from G1 showed normal histology of the kidney of rats. The proximal convoluted tubules, the distal convoluted tubule and the renal corpuscles with glomerulus and glomerular capsule are very clear and prominent. The renal corpuscle with visceral layer, parietal layer and capsular space is clearly observed. Animals treated with powder to G2 and G3 were not much affected. Treatment G (4) and (5) of kidney in animals fed with powder shows necrosis, vacuolation and disruption of the glomerular tubules. There were moderative degenerative changes and necrosis of the epithelia of the kidney tubules and glomeruli with both high doses (15 and 20 gm/kg body weight) of the herbal powder. Vacuolation was also noticed at both dosesG4 and G5; however this was not observed in low dose groups of G2 and G3 body weight.

The significant changes were observed in the levels of serum cholesterol and triglyceride levels in animals fed with low dose of ***G. sylvestre*** which may be due to the normal diet and normal rats as observed. In the higher concentration of G4 and G5 body weight 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. Thus the decrease in cholesterol may be due to faecal excretion of cholesterol which might be due to an interruption with the formation of micelles that contain cholesterol and bile acids in the gut and following interference with absorption of cholesterol.

Reduction in plasma cholesterol, triglycerides, and free fatty acid levels was observed in two studies of diabetic patients who received supplements of *Gymnema* 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 drugs. It should be noted that lipid lowering was a secondary endpoint in these studies, which were designed to demonstrate the antidiabetic effects of *Gymnema.*

Traditional experience may be of little help in the recognition of harmful interactions with conventional drugs[9,10]**.** Mortality and behavioral changes are considered as basic parameters to assess the toxicity of any herbal product.

G. sylvestre leaves have been widely used as health foods as the users often expect weight reduction and improvement of diabetes because of their ability to suppress the taste of sweetness and inhibit glucose absorption[8]**.**

In the present study body weight gain and food intake decreased in a dose dependent manner in the rats administred with *G. sylvestre*. The decrease in body weight gain may have been due to the decrease in food intake. Moreover these plant powder is known to suppress the receptor of taste of sweet on the tongue may be another cause**.**

Dose dependent changes were also observed 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 function of the animal and thus not posing severe toxicity that may lead to the death of the animal.

The leaves raise insulin levels, according to research in healthy volunteers[2], possibly due to regeneration of β-cells of the pancreas[3].

Toxic agents may affect the kidney and impair its physiological functions. These effects are detectable and/or quantifiable by cross checking the normally expected functions of the kidney in excreting the non-threshold nitrogenous waste products of metabolism like urea and creatinine. Also, by determining the ability of the kidney to filter and reabsorb the body-needed threshold substances like electrolytes, toxicity to the kidney may be detected[7]. Some authors have demonstrated that estimation of urea and creatinine levels is not sensitive enough in detecting a low level of renal toxicity or damage. Others have observed the absolute kidney weight to be a relatively sensitive indicator of nephrotoxicity for known nephrotoxicants. Nephrotoxicity has, therefore, been defined as increased kidney weight (either absolute or relative) coupled with a significant alteration in at least one serum parameter [6].

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*[11].

Animals treated with G2 and G3 were not much affected by ***G. sylvestre.*** Sections of kidney in animals fed with powder to G4 and G5 shows necrosis, vacuolation and disruption of the glomerular tubules. There were moderative degenerative changes and necrosis of the epithelia of the kidney tubules and glomeruli with both high doses (15 and 20 gm/kg body weight) of the herbal powder. Vacuolation was also noticed at both doses; however this was not observed in low dose groups of G2 and G3. Necrosis and cellular infiltration were noticed in some of the glomeruli and renal tubules of the rats in the high dose group.

The glomeruli, because they receive one-quarter of the cardiac output and are perfused at the highest pressure of any capillary bed in the body, are vulnerable to injury by a number of drugs and other toxic agents. These agents may lead to damage by one of two basic mechanisms: 1) direct, dose-related toxic injury; 2) indirect, immunologically mediated injury, largely dependent on dose.

Kidney from the control animals showed normal renal morphology. Because medicinal herbs are usually self-prescribed by the consumers, recommendations for the use of prescribed drugs like dose, manner, and frequency of administration, which are reviewed and controlled by the prescribing physician, is lacking. These factors increase the risk of toxicity of *Gymnema* as consumers use the extract for as long as their conditions last, and even at increased doses. Gymnema sylvestre leaf extract: a 52-week dietary toxicity study in Wistar rats[5].

In the kidneys, the histopathologic changes were that of acute tubular necrosis with diffused interstitial and glomerular haemorrhage. This suggests that irreversible cellular injury affecting the epithelial parenchyma and endothelial cells occurred. Kidney histology of treated rats showed features consistent with renal epithelial injury from toxins. Many herbal preparations have been found to exhibit renal tubular necrosis showing extensive interstitial fibrosis and severe tubular loss most prominent in the outer cortex[4] (Plate 1.).

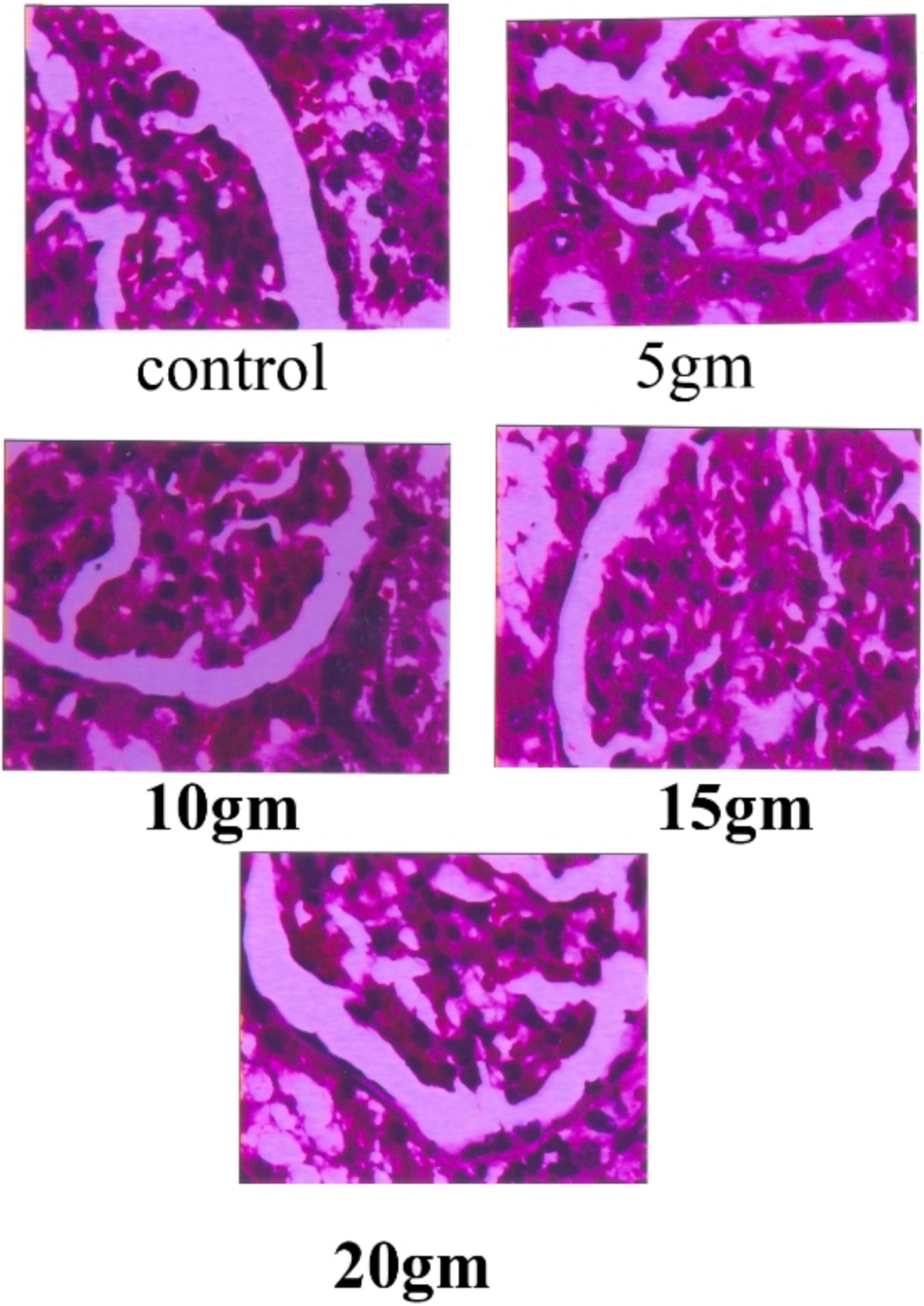

Plate 1.Histopathology in kidney

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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