IN-VIVO STUDY OF PROANTHOCYANIDINS HERBOSOMES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

This study shows the investigation of antidiabetic activity of proanthocyanidin in STZ-induced diabetic model rats. Proanthocyanidin ameliorated the diabetic condition by significant decreases of serum glucose, glycosylated protein, and serum urea nitrogen as well as decreases of urinary protein and renal-AGE in STZ-induced diabetic rats and decrease of serum glucose as well as significant decrease of glycosylated protein in type 2 diabetic mice. The suppression of ROS generation observed in the groups administered proanthocyanidin. Moreover, proanthocyanidin, especially its oligomeric form, affected the inflammatory process with the regulation of related protein expression, iNOS, COX-2 and upstream regulators, NF-κB, and the IκB-α. Moreover, expressions in the liver of SREBP-1 and SREBP-2 were downregulated by the administration of proanthocyanidins. The protective effect against hyperglycemia and hyperlipidemia in diabetic models was significantly strong in the groups administered. This suggests that oligomers act as a regulator in inflammatory reactions caused by oxidative stress in diabetes.

KEYWORDS: - anti-diabetic**,** proanthocyanidin, *in-vivo*

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1. INTRODUCTION

In modern era, Diabetes mellitus become the most serious problem world is facing. It may disturb the metabolisms. By this disturbance it may defect the secretion and

action of insulin. In most countries diabetes mellitus shows the complications and may affect on people and in socioeconomic challenges. Around 25 to 30% of population may affected.[1,2] Diabetes may be developed by genetic and environment factor. At the early stage of diabetes, the metabolism of sugar is been stopped and the insulin is not formed. This may result in lack of insulin and it may regulate the blood glucose level. This may break the fat, protein and glycogen and produces sugar. It led to the high sugar in blood and liver may release the ketones. Macromolecule's metabolism may be disturbed due to the distinguished in chronic hyperglycemia. This result into impairment of secretion and action of insulin.[3,4] This damage may occur for long term. This disease may also damage or failure of heart, eyes and nerves. It may lead to disability and death. Hyperglycemia may damage the organ system and may relate the diseases how long it been present and how it may be controlled. Diabetes may also cause polyuria, weight loss and thirst.[5,6] Herbosomes technology improve the bioactivity of plant extracts and act as bridge between NDDS system and traditional system. It is a complex of phytoconstituents and lipid substances which enhance permeation of plant extract. Herbosomes are recent advanced novel drug delivery system of herbal formulations that have enhanced released rate and bioavailability than conventional dosage forms. Since they have improved pharmacological and pharmacokinetic parameters, they can be used in the treatment of the acute and chronic liver disease.[7,8,9]

Fruits of *Vitis vinifera* have been used for thousands of years because of their nutritional and medicinal benefits. They are rich in sugars, flavonoids, anthocyanins and proanthocyanins, organicacids, tannin, mineral salts and vitamins. Grapesskin, especially from there and black species is rich in resveratrol which is a derivative of stilben. Studies have shown that resveratrol is one of the strongest known natural antioxidants. It is found in a large quantity in black grape juice, skin and seed. The seeds and the leaves of the grapevine are used in herbal medicine

and its fruits are utilized as a dietary supplement.[10,11,12]

2. MATERIAL AND METHODS

2.1 Preparation of extract

2 kg of fresh *Vitis vinifera* (without drying) were instantly blended with 5L of water in a mixer, squeezed by hand and filtered through muslin cloth to yield the aqueous extract. Solvents used were petroleum ether, chloroform, ethyl acetate, n-butanol, and water. After that the sample is transferred into the rotatory vacuum evaporator with 40^0C temperature until the solvent has been evaporated. After that these sample were kept into the refrigerator at 4^0C. these are then used for the phytochemical analysis. Sample has been weight and stock solution were prepared. These solutions are used for the further analysis.

2.2 *In vivo* study

2.2.1 Animals

Male albino rats (Wistar strain) weighing 200–220 g was used for this study. Animals were housed in groups of six in colony cages at an ambient temperature of 20–25°C and 45–55% relative humidity with 12 h light/dark cycles. They had free access to pellet chow and water *ad libitum*. The experiment was performed with the ethical guidelines as per the protocol approved by Institutional Animal Ethics Committee CPCSEA.

2.2.2 Preparation of solutions

2.2.2.1 Preparation of solution of SE: 100 mg of SE was accurately weighed and dissolved in 5 mL of distilled water to yield a solution of 20 mg/mL.

2.2.2.2 Preparation of dose of formulation F8: 153 mg of herbosomes F8 (weight equivalent to 100 mg of SE considering the percent loading of Proanthocyanidins) was accurately weighed and mixed to yield a homogenous mixture having equivalent of Proanthocyanidins as the formulation of SE was prepared.

2.2.3 Herbal formulation tolerance test

This test is performed by fasting of animals by overnight for 18hs.

- Group I control groups
- Group II had glucose control rats

- Group III administration of Glibenclamide (0.5 mg/kg)
- Group IV rats are injected by SE (100 mg/kg)
- Group V rats are injected by SE (200 mg/kg)
- Group VI rats are injected by Herbosomes F8 (100mg/kg)
- Group VII rats are injected by Herbosomes F8 (200mg/kg)

In group II to group V, glucose was fed to the rats 30minutes before the administration of extract and drug (Glibenclamide). From the retro-orbital sinus the sample were collected from 0minutes, 30minute and 90 minutes of administration of extract and drug (Glibenclamide). The blood plasma was collected and it was centrifuged at a speed of 3000RPM.

2.2.4 Induction of NIDDM

In this experiment around 170 to 200g of male albino rats were taken and remain fasting for overnight by single injection of streptozotocin (60 mg/kg) administration 20minutes after administration of nicotinamide (120 mg/kg). Streptozotocin and nicotinamide was dissolved in citrate buffer. The glucose is elevated in plasma with the confirmation of Hyperglycaemia. It was determined at 3 days and then after 7 days after the administration of injections. The threshold value is >126mg/dl in fasting plasma. For this study only those rats were taken who shows NIDDM.

2.2.5 Evaluation of antidiabetic activity of Proanthocyanidins-phospholipidcomplex

For performing these experiments, seven group is made and in each group contains 6 rats each. For 28days the prepared herbosomal formulation was administered.

- Group I control group
- Group II had diabetic control rats
- Group III administration of Glibenclamide (0.5 mg/kg)
- Group IV rats are injected by SE (100 mg/kg)
- Group V rats are injected by SE (200 mg/kg)
- Group VI rats are injected by Herbosomes F8 (100mg/kg)
- Group VII rats are injected by Herbosomes F8 (200mg/kg)

After Administration of extract the fasting glucose level are determined on 0, 7th, 14th and 28th days. In the experimental period, selected animals were weighted and the change in weight is also monitored and calculated.

2.2.6 Toxicity study

2.2.6.1 Acute oral toxicity study

Study of acute toxicity of formulation, wistar albino rats are use and procedure according to OECD.

- Group I control
- Group II rats are injected by F8 (250mg/kg body weight)
- Group III rats are injected by F8 (500mg/kg body weight)
- Group IV rats are injected by F8 (1000mg/kg body weight)
- Group V rats are injected by F8 (2000mg/kg body weight)
- Group VI rats are injected by F8 (4000mg/kg body weight)

Overnight fasting the animals only water was given after the administration of single dose of formulation of F8. After 4h of administration of drug no food and water are given to the animals. After every 30min toxicity of drug and mortality of animals were studied. It was done for 24h and up to 14 days. If the animals show any kind of mortality the drug dose is toxic. In any animals no mortality was shown then the treatment of animals with higher dose. Different parameter is determined after the administration of drugs in 30minutes for first 24h and then it was continuous for 14days.

2.2.6.2 Repeated oral toxicity study

In this experiment animals were divided into 6 groups with 10 animals each (5 males and 5 females).

- Group I control
- Group II rats are injected by F8 (250mg/kg body weight)
- Group III rats are injected by F8 (500mg/kg body weight)
- Group IV rats are injected by F8 (1000mg/kg body weight)
- Group V rats are injected by F8 (2000mg/kg body weight)
- Group VI rats are injected by F8 (4000mg/kg body weight)

For 28days the toxicity was observed with different parameters like mortality, body weight, food and water. After the completion of experiment the animals were sacrifices by sinus puncture and dislocation of cervical. for histopathologic analysis, the organs were separated and it was stored in a 10% formalin solution.

2.2.6.3 Biochemical parameters estimation

These parameters are determined on the 12^{th} days of sacrifice by dislocation of cervical. by using auto-analyzer glucose oxidase method is use to determine the LDL, HDL, TGL and total cholesterol.

2.2.6.4 hematological parameters determination

Automatic analyzer is use for the determination of WBC, RBC, neutrophile, lymphocyte and monocyte.

2.2.6.5 Plasma biochemical parameters determination

In biochemical parameter, different parameter (SGOT, SGPT, ALP, Triglyceride etc) were determined by standard techniques of chemical analyzer.

2.3 Histopathological evaluations

By using light microscope autopsy is done. It was done for histopathological studies. The tissue sample were taken from liver and kidneys. This neutralizes by 10% buffers and done at room temperature. For 24h the fixation of specimens is done. These tissues were washed to a running water and dehydrated by ethyl alcohol. It was then clean with xylene, impregnated with wax in oven and it was embedded in paraffin at room temperature.

The microtome is 5-6µ thick and paraffin block it. 8 to 10 sections of ribbons are collected and it was floated in flotation bath. The temperature is set to 40°C. after that these tissues are put on microscopic slides. The slides were rinsing with Harris haematoxylin for 10 to 15minutes. For the removal of excess stains 1% acid alcohol is use. After that the excess acid and destain halt were removed by putting it into the running tap water. Sodium bicarbonate is use for placing of slides and 1% alcoholic eosin is for counterstained for 2min. By increasing the concentration of ethanol dehydration of H & E-stained sections. It was clear by xylene and using DPX

mountant and glass cover slips are mount on the slide.

3. RESULT AND DISCUSSION

3.1 *In vivo* study

3.1.1 Antidiabetic activity of herbosome formulation

Herbosome formulation can be used to create increasingly evident therapeutic sufficiency over time. Individual plants do not contain enough active compounds to produce the desirable and beneficial effects that are desired. Whenever the numerous plants are combined to a specific extent, this will have a greater beneficial effect and will reduce the toxicity of the combination.

3.1.2 Formulation tolerance effects on glucose

In control group the rise in glucose level was observed. In drug(glibenclamide) treated group there is a decrease in plasma glucose level. The herbal formulation showed glucose tolerance efficacy. The F8 (herbosomal formulation) has greatest glucose tolerance properties compared to other formulation.

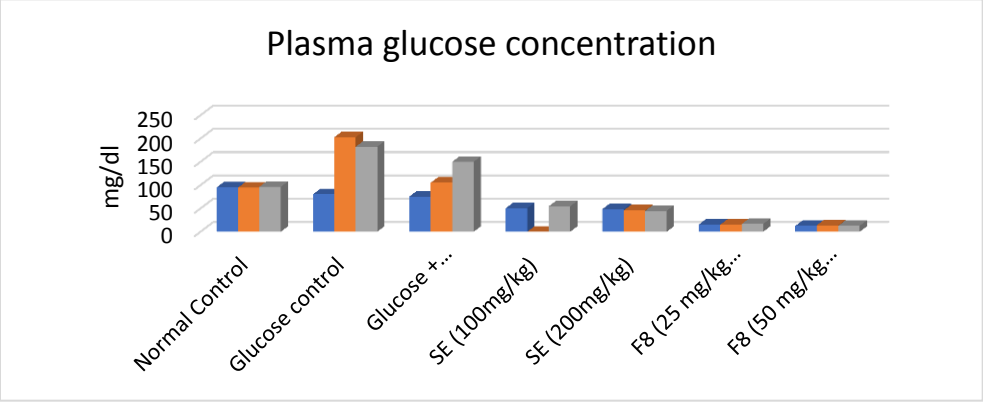

Figure 1. Plasma glucose concentration of herbosomal formulation

3.1.3 Formulation effects on NIDDM

Due to the high fasting the induction of diabetes in experimental rats are confirmed. In streptozotocin control groups there is an increase in glucose level were recorded from 0 to 28th days. Those animals which were treated with the drug (glibenclamide) decrease in serum

glucose level. Those animals which are treated with herbosomal formulation (F8) show decrease in glucose level. From the formulation it was concluded that the formulation F8 show good antidiabetic activity.

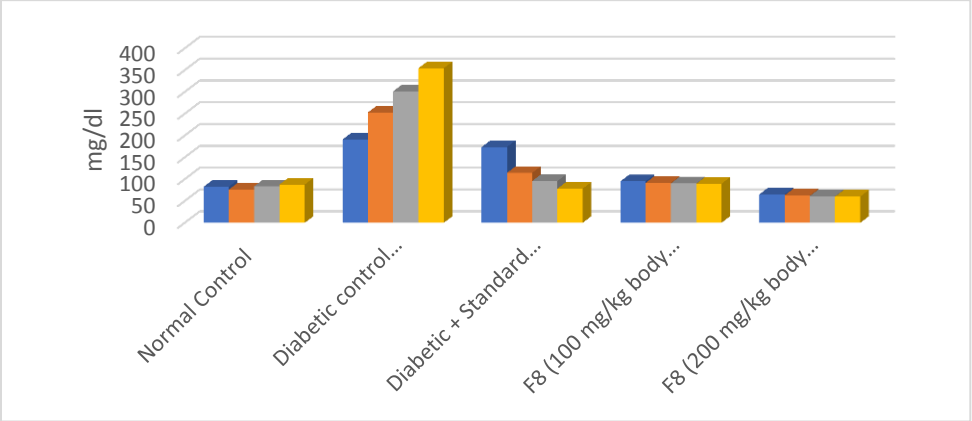

Figure 2. Herbosomal formulation effect on diabetes mellitus

3.1.4 Hyperlipidaemic activity of herbosomal formulation

The lipid profile is exhibited in control and experimental rats. In diabetic control group animals show high TGL, LDL, HDL and total cholesterol. In group treated with drug (glibenclamide) show decrease in TGL, LDL, HDL and total cholesterol. Those animals which are treated with herbosomal formulation shows decrease in TGL, LDL, total cholesterol but there is a slightly increase in HDL. This observation is done on 28th day of experiment. The herbosomal formulation F8 shows better and maximum antihyperlipidemic activity.

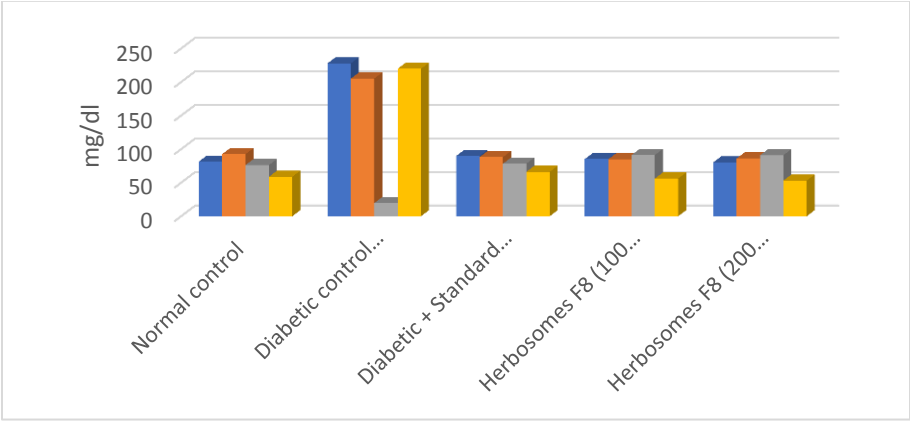

Figure 3. Anti-hyperlipidaemic activity of herbal formulation

3.1.5 Effect on body weight

During the experiment each and all rats were weighing before and after introduction of diabetes. There is a decreased in body weight after the induction of diabetes and after the introduction of herbosomal formulation the body weight is increases.

Group	Change in Body weight (gm)		
	Before Induction	After Induction	After Treatment
Normal control	188.2±2.7	196.4±4.6	182.7±1.8
Diabetic control (Streptozotocin)	182.1±4.1	125.6±2.8	102.4±3.5
Diabetic + Standard Glibenclamide (0.50 mg/kg)	190.3±5.6	144.7±8.2	191.4±2.4
Herbosomes F8 (100 mg/kg body weight)	179.5±2.5	185.2±3.2	184.7±2.7
Herbosomes F8 (200 mg/kg body weight)	186.3±3.9	195.2±1.8	189.1±4.5

Table 1. Effect on body weight

3.2 Toxicity study

The findings of antidiabetic study suggested, F8 having higher antidiabetic and antihyperlipidemic activity compared to other formulation. To develop herbosomal formulation with better safety and high efficacy, necessary to understand the toxicity profile of the formulation. It is because on mixing the various plant material, the phytoconstituents can interact with each other, and these interactions can affect the efficacy of herbosomal formulation. The toxicity evaluation of F8 was performed to determine its safety.

3.2.1 Acute oral toxicity study

Results show that there is no toxic symptoms and mortality were shown by rats. It was done up to 4000mg/kg up to 14 days. There are no changes were observed during visible testing. The herbosomal formulation F8 show no changes and mortality in any parameters.

Observation	Control	250 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	4000 mg/kg
Body weight	No	No	No	No	No	No

Temperature	No	No	No	No	No	No
Food intake	No	No	No	No	No	No
Urination	No	No	No	No	No	No
Rate of respiration	No	No	No	No	No	No
Change in skin	No	No	No	No	No	No
Drowsiness	No	No	No	No	No	No
Eye color	No	No	No	No	No	No
Diarrhea	No	No	No	No	No	No
Death	No	No	No	No	No	No

Table 2. Acute toxicity study

3.2.2 Repeated oral toxicity

Results shows that there is no mortality and intoxication were observation.

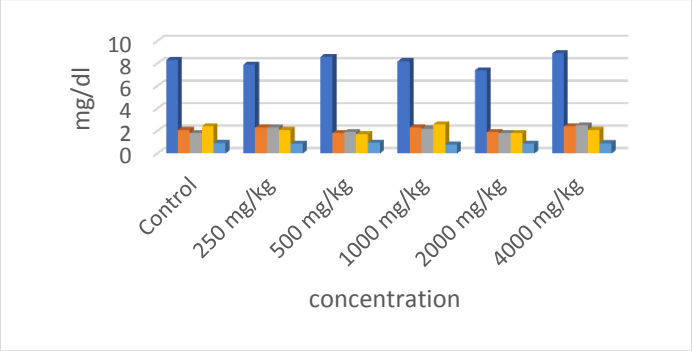

Figure 4. Oral administration effect of F8 on organ weight (g) of rats

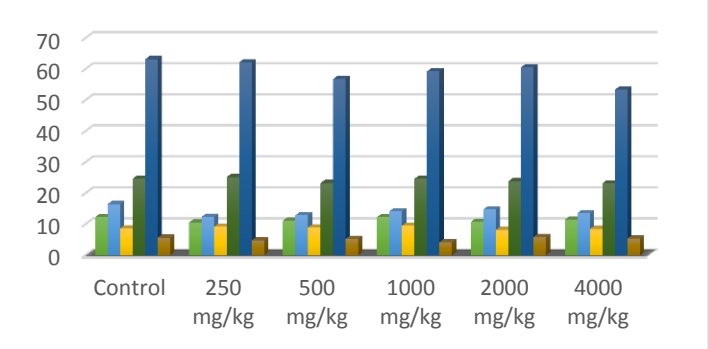

Figure 5. Sub-acute toxicity of F8 in Haematological values of rats

3.3 Histopathological evaluations

After 28th day of the experiment histopathology of the liver and kidney were observed of groups treated with herbosomal formulation (F8).

3.3.1 Histopathology of Liver

The liver section of rats treated with herbosomal formulation (F8). It observed that there is a normal appearance with endothelial and Kupffer cells. The size and shape of the hepatocytes are normal and in cytoplasm no vacuoles are observed.

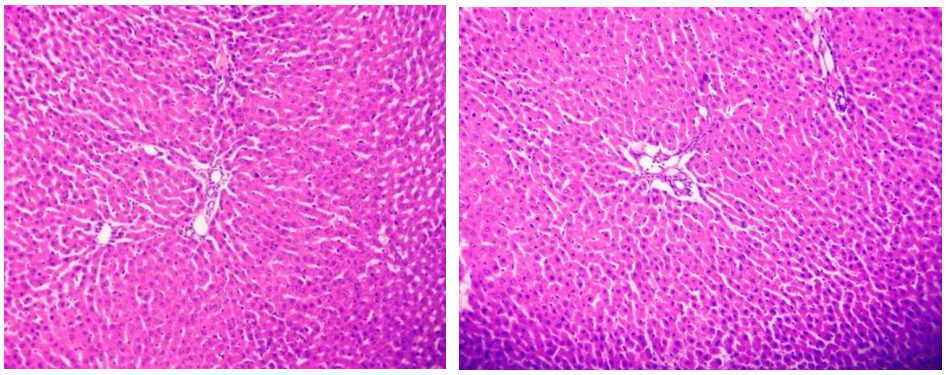

Figure 6. Microscopic feature of liver showing normal hepatic cells and central vein

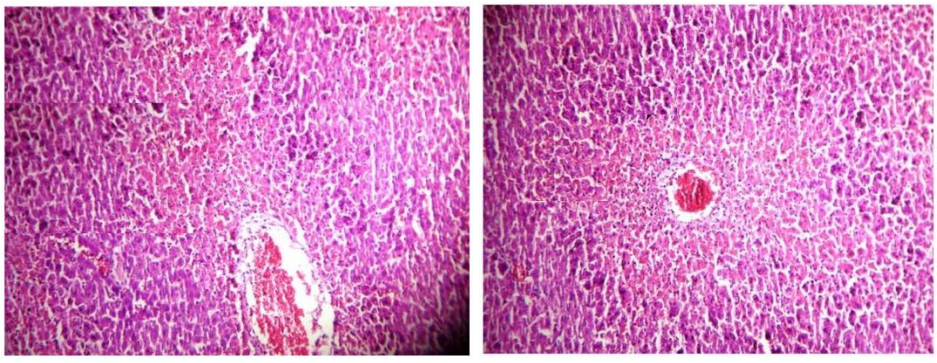

Figure 7. Microscopic feature of liver showing normal central vein and no significant changes in hepatic cells

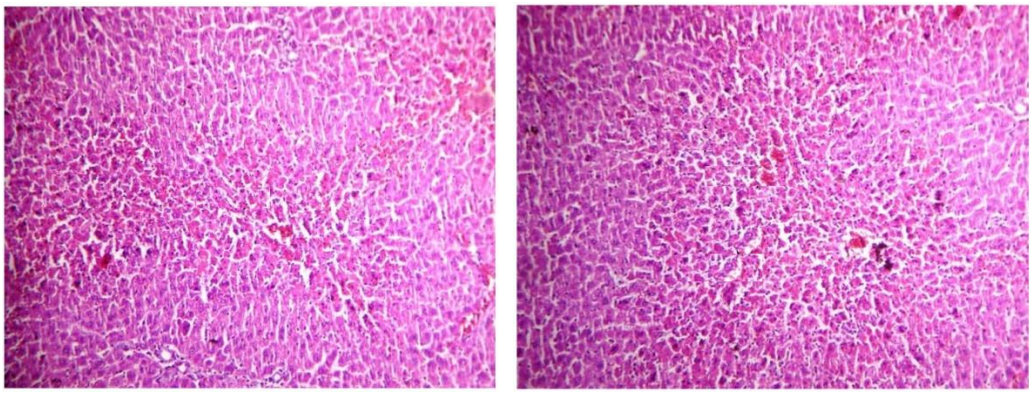

Figure 8. Microscopic feature of liver and hepatic cell showing haemorrhage and dilation of sinusoids.

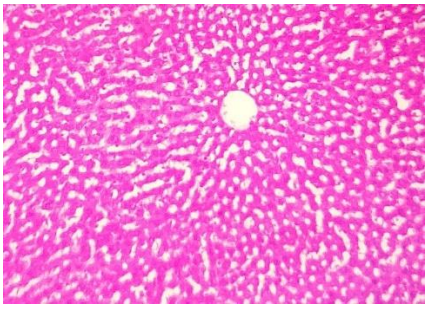

Figure 9. Microscopic feature of hepatic cell from central vein.

3.3.2 Histopathology of Kidney

The kidney section of rats is treated with herbosomal formulation (F8), shows no histopathological changes. Observation shows that the bowman's space show normal appearance. After administration of herbosomal dose F8 everything is normal.

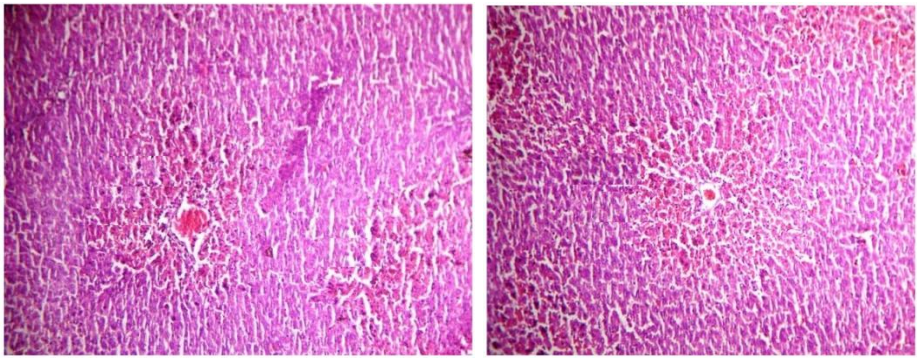

Figure 10. Microscopic feature of kidney showing normal structure of renal tubules and bowman's capsule.

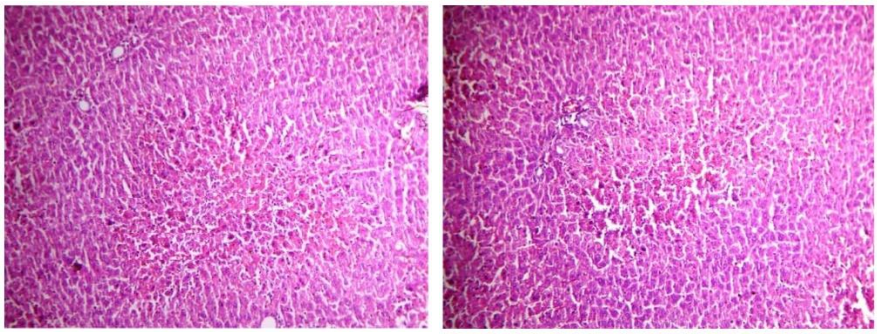

Figure 11. Microscopic feature of kidney showing normal structure proximal and architecture of renal and distal tubules with normal cubic epithelium cells and bowman's capsule.

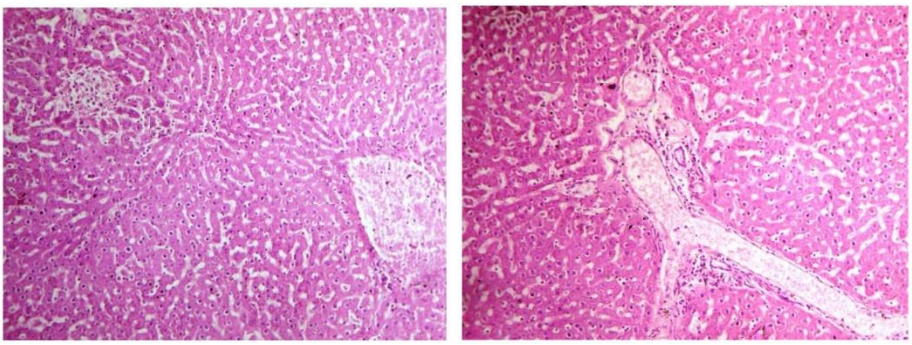

Figure 12. Microscopic feature of kidney showing normal histology of proximal distal and renal tubules. No changes in glomeruli.

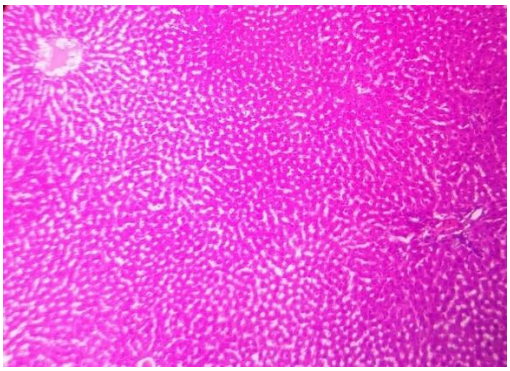

Figure 13. Microscopic feature of kidney showing accumulation of serous fluid in the few renal tubules.

4. SUMMARY AND CONCLUSION

In toxicity study, the herbosomal formulation F8 shows no toxicity and no mortality if the dose is rising up to 4000 mg/kg body weight. There were no changes in the body weight, temperature, food intake, urination, rate of respiration, change in skin, drowsiness, eye color, and diarrhea.

In the repeated oral toxicity study, there was no significant change in organ weight, hematological parameters (RBC count, WBC count, Neutrophil, Lymphocyte and Monocyte), and biochemical parameters in F8 treated rats 28 days and the results are almost similar to control animal.

No histopathological changes observed in rats treated with F8. In any treated groups did not has any hepatoxicity. So that F8 show non hepatotoxic. In histological examination of kidney, the herbosomal formulation F8 show no such changes. The herbosomal formulation F8 incorporating different ratio of the *Vitis Vinifera* were prepared for the evaluation of the antidiabetic activity. From result it was concluded that the herbosomal formulation F8 is very much safe up to the higher dose of 4000mg/kg. Herbosomal formulation show the antidiabetic activity for diabetic society. It also shows the scope for clinical trial on humans.

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