

HEPATOPROTECTIVE AND ANTIDIABETIC EFFECTS OF *NIGELLA SATIVA* SEED EXTRACT IN ALLOXAN-INDUCED DIABETIC MICE: AN EXPERIMENTAL STUDY WITH HISTOPATHOLOGICAL INVESTIGATIONS

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Article Received on 10/07/2017

Article Revised on 30/07/2017

Article Accepted on 20/08/2017

ABSTRACT

Objectives Diabetes Mellitus (DM) is known to induce oxidative stress causes liver tissue injury and changes the homeostasis of carbohydrate, lipid and protein metabolism. Hence, present study is aimed to investigate the hypoglycemic and hepatoprotective activities of the *Nigella sativa* (NS) against experimentally induced diabetic mice. **Methods** Experimental micewere divided into five experimental groups (n=6).DM was induced by a single intraperitoneal (i. p.) injection of alloxan (80 mg/kg body weight). Fasting blood glucose (FBG) and body weight (BW) were measured in the particular days (1, 8, 16, 24 and 32) of treatment period. The histological changes were examined by hematoxylin–eosin (HE) staining. Liver function markers and lipid profile were also measured by using enzymatic kits. **Key Findings** Treatment with NS (50 and 200mg/kg BW) markedly increased BW gain and significantly ($p < 0.001$) reduced FBG level compare to diabetic mice. Histopathological examination showed that the NS partially recovered hepatic injury. SGPT, SGOT and ALP levels were found to decrease ($P < 0.01$) in NS treatment groups. Hepatic dysfunctioning was found strongly correlated ($P < 0.01$) with diabetes. **Conclusion** Methanol extract of NS possess hypoglycemic and hepatoprotective properties. Therefore, NS could be used as an alternative medicine in management of diabetes.

KEYWORDS: Diabetes Mellitus, Histopathology, Hypoglycemic, Hepatoprotective, Fasting blood glucose, Lipid profile.

INTRODUCTION

The liver is a vital organ that plays central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of continuous supply of glucose in organs that are largely dependent on glucose as their energy source.^[1] But diabetes makes the liver become malfunctioning and considering it as the most common cause of liver disease.^[2] Virtually the entire spectrum of liver diseases including abnormal liver enzymes, nonalcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, hepatitis C and acute liver failure are seen in patients with DM^[3] Thus, the prevalence of liver disease is high in DM and vice versa.

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.^[4] It associates with microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications leading to high

diabetic morbidity and mortality.^[5] In pharmaceutical practice, lifelong insulin therapies and oral hypoglycemic agents are generally used for treatment of diabetes. However, regarding the fundamental challenges of glycemic control along with hepatoprotection, the versatile application of these medications remains unsatisfactory due to claim with acute adverse effects including hypoglycemia, gastrointestinal disturbances, renal toxicity, hepatotoxicity and poor adherence to treatment.^[6] Therefore, alternative medicines are needed to achieve glycemic control upon preserving the liver.

In the last few years, herbal medicines have attracted the attention of researchers for treatment of various diseases including Diabetes Mellitus due to having their less potential side effects. The World Health Organization (WHO) has listed 21,000 plants, which have great medicinal values and have been used for various purposes around the world. Many of them have been known to possess hypoglycemic activity and generate natural antioxidant.

Nigella sativa (NS) seed (commonly known as black cumin) is a plant commonly found in Asian, Mediterranean, Middle Eastern, and African countries, and its belong to the Ranunculaceae family.^[7] NS is traditionally used for the treatment of anti-viral, anti-inflammatory, anti-diabetic, immunomodulatory, anti-cancer and hepato- protective activities.^[8,9] In addition, NS seed oil is used to lessen the toxic effects of several chemotherapeutic agents.^[10]

Herein, we evaluated the hypoglycaemic and the hepatoprotective effect of *N. sativa* methanol extract in alloxan induced diabetic in mice. The possible reasons for hepatic dysfunctioning in diabetic statuses were also examined through histological studies of mice liver.

MATERIALS AND METHODS

Plant material and extraction procedure

The NS seeds were purchased from a local market in Rajshahi, Bangladesh. After washing seeds were dried and grounded into coarse powder by grinding machine. Then, 300 gm of dried powder were taken in amber colored extraction bottles (2.5 liter capacity) and seed materials were soaked with methanol (500mL × 3 times). After sealing bottles were kept at 7 days with occasional shaking and stirring. The extract was filtered through cotton and Whatman No.1 filter papers followed by concentrated with a rotary evaporator under reduced pressure at 45°C to afford crude methanol extract.

Chemicals

Alloxan was purchased from Sigma chemical Co. Other chemicals were purchased from Merck Company (Germany). All others chemical were used in analytical grade.

Animals

Swiss-albino mice of both sexes, average weighing 28-32 g were purchased from International center for diarrhea disease research Bangladesh. The animals were kept in individual propylene cages under standard laboratory conditions. Standard room conditions including 12 hour light/dark cycle at 22±1°C and 50±10% humidity had been maintained and the animals were fed with standard mice diet and water ad libitum. All animals were guided according to the guideline prepared by the National Academy of Sciences namely "Guide for the Care and Use of Laboratory Animals" and published by the National Institutes of Health.

Experimental design

Mice were divided into five groups (n = 6 in each group). Diabetes was induced in all groups except the normal control group by a single intraperitoneal (i.p.) injection of alloxan (80 mg/kg) freshly dissolved in 5 mmol/L citrate buffer (pH 4.5), as described previously.^[11] Three days after alloxan injection, blood from the tail vein were used to measure the glucose level by using a One Touch Glucometer (Life scan; Johnson & Johnson, New

Brunswick, NJ, USA) for making conformation of diabetic causation. Mice with blood glucose levels over 15mmol/L were considered diabetic. Detailed descriptions of the five groups are as follows: Group A (normal control group), mice were injected with an equal volume of vehicle (citrate buffer); Group B, (diabetic control) untreated alloxan diabetic (80 mg/kg b.w., IP); Group C, treated alloxan diabetic with methanol extract of NS (50 mg/kg b.w. IP); Group D, treated alloxan-diabetic with methanol extract of NS (200 mg/kg b.w., IP) and Group E (positive control) treated alloxan-diabetic with standard glibenclamide (50 mg/kg b.w., IP), and 32 days were evaluated to assess its effect on fasting blood glucose (FBG), and in different groups fasting blood glucose (FBG) and body weight (BW) were measured in the particular days (1, 8, 16, 24 and 32).

In vivo Toxicity study

Healthy swiss-albino mice of either sex (equal number of male and female mice weighing 28-32 g) fasted for 12 hours were divided into drug-treated 'test' groups and vehicle-treated [1% CMC (Carboxy methyl Cellulose)] 'control' group, totally making up five groups of four mice each. The methanol extract of NS [250, 500, 1000, 2000 mg/kg body weight (b. wt.)] were separately administered to the mice in each of the test groups, while the control group was administered with 1% CMC, to evaluate the toxic effects produced on liver and kidney. Further the mice in both the test and control groups were provided access to food and water, and gross behavioral changes were observed over a period of 7 days for signs of acute toxicity.^[12]

Measurements, tissue preparation, biochemical assays and histological studies

The body weight of the mice in all groups was recorded before the start of the study and seven days interval during the experimental period. Blood glucose analysis (with the help of Gluco Check) was done seven days interval on overnight fasted animals. After 32 days, the mice were sacrificed after overnight fast under Sodium Pentobarbitone (10 mg/kg). The blood was collected by cardiac puncture in a tube and allowed to clot for 30 min at room temperature. The tube were centrifuged at 1000 × g for 15 min and the serum was separated, and stored as 0.5 ml aliquots in Eppendorf tubes at -80 °C for later analysis.

Plasma lipid profile such as triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) levels were determined by enzymatic methods, using commercially available kits (Linear chemicals, Barcelona, Spain). The low-density lipoprotein-cholesterol (LDL-C) fraction and very low density lipoprotein-C (VLDL-C) were calculated according to the formulas below as described by William *et al.*^[13]

$$\text{LDL-C} = \text{HDL-C} + \frac{\text{TG}}{5} - \text{TC}$$

$$\text{And, VLDL-C} = \frac{\text{TG}}{2.2} \text{ (mmol/L)}$$

Subsequently, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were analyzed using diagnostics kit (Linear chemicals, Barcelona, Spain). The liver was dissected, immediately rinsed with ice-cold saline and immediately frozen and stored at -80°C for further histopathological analysis.[14] At the time of histological analysis liver specimen were cut into smaller pieces (around 1 mm X 1 mm X 1 mm), fixed with formalin solution (10%) and instantaneously processed for histopathological studies by paraffin method.[15] Briefly, the sections were processed by passing through different mixtures of ethyl alcohol and water (45, 75, 95% and finally incubated in alcohol) for dehydration, cleared in xylene and embedded in paraffin. Further, 50 μm sections of the tissues were obtained using rotary microtome, stained using hematoxylin-eosin (HME) dye and mounted in deparaffinised xylene medium for microscopic observation.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0. Data were presented as mean \pm standard error of the mean (SEM). Significant results were marked. P value was considered significant if $P < 0.05$.

RESULTS

Acute toxicity of *N. Sativa* on experimental mice

Oral administration of NS to animals up to a dose of 2000 mg/kg BW did not exhibit any amendment in their behavioral pattern, physical parameters and no animal was found dead up to 7 days. Further, the animals neither produced any signs of toxicity nor mortality symptoms, thus illustrating the non-toxic nature of NS. Therefore, further investigation of activity was carried out using 50 and 200 mg/kg dose levels.

Effect of *N. Sativa* extract on body weight

Effect of methanol extract of *N. sativa* on body weight in alloxan-induced diabetic mice was summarized in the Table 1. The changes of mice body weights in five groups were investigated. There was a progressive increase in the body weight in the control and a progressive decrease in the alloxan group, whereas the body weight in the groups 3, 4 and 5 showed a progressive decrease in body weight from 1-16th day's administration on NS extract, but after 16th day's progressive increase was found. Administration of NS attenuated the weight loss in alloxan induce diabetic mice. In the present experimental condition, the treatment of alloxan + NS had significant ($P < 0.05$) effect on changes in body weight. The mean BW of animals in group 3, 4 and 5 at the 16th day of this study were 28.53, 26.54 and 28.13gm respectively while at 32th day of the study they were found to be 31.42, 29.83 and 30.02gm respectively.

Effect of *N. sativa* extract on blood glucose level

The plant extract produced significant changes in the blood glucose level in alloxan-induced diabetic mice. Comparing the blood sugar level in diabetic mice group, plant extract administered groups showed significant ($P < 0.001$) reduction of blood glucose which was as near as glibenclamide administered group (figure 1). In 8th to 32th days, plant extract administration groups maintained glucose levels 6 % - 45% lower than the diabetic control group.

Effect of *N. sativa* extract on blood lipid profile

Table 2 showed the serum levels of TC, TG, LDL-C, HDL-C and VLDL-C in normal and experimental animals of each group. A significant ($p < 0.01$) reduction in TC and TG (by 24.97-29.84% and 26.43-37.05% respectively) were evident in the treated group when compared to the diabetic control mice. Where in positive control group glybenclamide cause the reduction of TG and TC were respectively 41.93 and 33.10%. The result was significantly similar with treated group (200mg/kg), supported the extract efficacy. On the other hand in lipoprotein count diabetic control group showed highest LDL and VLDL-C count according to lowest HDL content. The higher dose (200mg/kg) of NS significantly lessened the increase in the level of LDL and VLDL-C (4.53 and 0.53 mmol/L, respectively) compared to diabetic control, while treatment of diabetic mice with 50 mg/kg dose less effective in attenuating the increases in either cholesterol in serum. HDL-C level in serum for treated group were evidenced to increase 5.1-9.7%, less significant from positive control group (34.58%).

Effects of *N. sativa* extract on liver function marker enzymes

There was a significant ($P < 0.001$) increase of SGPT and SGOT level after diabetes induction which was compensated by NS extract significantly ($P < 0.001$). The percent lowering of SGPT level by NS extract from diabetic control groups were 15%-20.19%, whereas 32.70 % for glibenclamide treated group. The reduction of SGOT level was highly significant ($P < 0.001$) for NS extract at 19.76-24.7% (figure 2). ALP levels were higher in diabetic control group than the normal group. ALP significantly reduced 33.50-39.31% with the treatment of NS extract compare to diabetic group, whereas glibenclamide reduce 45.22% of serum ALP level.

Histopathological findings

Liver histopathology of the animal of the control group didn't show any histological changes during the period of the experiments, the hepatic lobules were seen normally with polygonal hepatocytes having regular nucleus and cytoplasm. Normal central vein and normal sinusoidal spaces confirm the control mice liver integrity. In the diabetic animals, the liver showed several alterations including high degree of fatty changes, cloudy swelling, infiltration of lymphocytes with hemorrhage. Congestion in portal vessels & sinusoids with mild centrilobular hepatocyte

degeneration were also common in diabetic control group. Necrotic cell distortion also evidence for hepatic lesion in diabetic group. In histopathological observation of NS treated diabetic liver showed mild hepatic distortion with central vein shrinkage, enlarge sinusoids, mild fat deposition in fatty granules, inflammatory infiltrate in the portal tract etc.

Correlation between diabetic progressions with hepatic dysfunction

Liver function enzymes were significantly correlated ($P < 0.01$) with diabetic progression. They were positively correlated. Diabetic leads to the distortion of liver from their normal function and high value of liver function enzyme was the evidence for this abnormalities. Therefore increasing the blood glucose level in diabetic condition fascinating the induction of liver function enzyme in serum and high serum level of these enzymes are predominant marker for hepatic dysfunction.

DISCUSSION

While hyperglycaemic condition is known for its increased risk of liver disorders due to its direct implications on the blood lipid profiles and hepatic injuries, it is also held responsible for several other disorders collectively known as 'diabetic complications'.^[16] Traditional medical practices have advocated the control of several disorders by dietary modulation which definitely is the best approach even for the management of diabetes. Thus, in searching of natural therapy, hypoglycemic and hepatoprotective effects *N. sativa* methanol extract has been evaluated in the present study.

In our study, the untreated diabetic group was showed significant weight loss while treatment with NS at different doses (50 and 200 mg/kg b.w.) facilitate to increase BW gain in alloxan induce diabetic mice. This finding is in line with Kanter *et al.*^[17] described that NS remarkably improved BW gain in STZ-induced diabetic rats. A possible mechanism for this might be that NS reduces hyperglycemia and therefore protein wasting due to inaccessibility of carbohydrate does not occur.^[17]

In present study, the methanol extract of NS at doses of 50 and 200 mg/kg b.w. revealed a significant hypoglycemic effect in alloxan-induced diabetic mice by diminishing the fasting blood glucose (FBG) levels. In addition, results showed time dependent anti-hyperglycemic nature of the NS extract. This observation is in agreement with Farar *et al.*^[18] The mechanism of the hypoglycemic effect of NS has been suggested previously to be due to pancreatic actions via enhancing insulin secretion and inducing β -cell proliferation and regeneration [19,20]. Another group of researchers have proposed that NS enhancing tissue sensitization to insulin, especially liver and muscles and thus induced insulin dependent metabolism of glucose.^[21,22]

Elevated level of lipid in serum termed as dyslipidemia are directly associated with diabetes mellitus where the metabolic syndrome is characterized by central obesity, hypertriglyceridemia, low plasma HDL-cholesterol, hypertension and dysglycemia, i.e. impaired fasting glucose.^[23] In diabetic condition hypertriglyceridemia and hypercholestermia are the common factors involved in the development of the liver diseases including hepatitis- C, non-alcoholic fatty liver disease, acute hepatitis, liver cirrhosis and so on.^[24-27] Liver is the vital organ associated in the synthesis, secretion, catabolism, and storage of lipids and lipoproteins. Therefore, the abnormal serum lipids and lipoproteins concentrations predominantly indicate the causation of liver diseases.^[28,29] Deregulation of fat metabolism in the liver is accompanied by overproduction of very-low density lipoproteins (VLDL), the characteristic lipoproteins of the metabolic syndrome.^[30] On the other hand, lipid accumulation in hepatocytes might be developed due to the resistance of insulin.^[30] In non-alcoholic liver cirrhosis, HDL-cholesterol concentration diminished with the severity of disease, Ramcharran and co-workers^[31] have found that higher lipid levels except TG levels (that are directly related to steatosis) are associated with serious liver disease causation. Thus it is important to ameliorate the serum LDL, TG and TC level in diabetic condition to overpass the liver complications. Interestingly, NS reduced both serum TC and TG levels and increased HDL-C Level. The more prominent effect of NS extract is the reduction of LDL-C, which is a triggering factor for coronary occlusion and liver cirrhosis. This finding is agreement with the investigation of Asgary *et al.*^[32] describe that NS contains antioxidants such as tocopherols, phytosterols, and polyunsaturated fatty acids that can contribute to cholesterol reduction and prevention of cholesterol oxidation. Considering NS effect on these lipid components, it can be assumed a potential hypolipidemic agent, which will be a great advantage both in diabetic condition as well as the associated hyperlipidemic conditions.

The present study also demonstrated the effect of NS on liver function enzymes (SGPT, SGOT and ALP), where both of the parameters were significantly ($P < 0.01$) reduced in NS treated mice group, indicate the hepatoprotective potentiality NS. Because the elevated serum levels of these enzymes are directly associated to the diseases in liver. The SGPT and SGOT levels are also found to be increased in almost all liver diseases. Baxter & Schofield reported an increase in SGPT and SGOT in diabetics.^[33] Highest levels of alkaline phosphatase occur in cholestatic disorders is a condition where bile cannot flow from the liver to the duodenum cause hepatic injury.^[34] As markers of the liver functioning SGPT, SGOT and ALP enzyme activities are elevated during diabetic state which signifies the toxic effect of alloxan on liver.^[35]

On the other hand the present study showed liver disease markers were strongly correlated ($P < 0.01$) with diabetic

progression. These results indicated the adverse effect of diabetic on liver. Liver histological studies were also revealed the same effects of diabetes on liver. The present study showed the histological changes of liver in diabetes group. Where diabetes liver showed progressive changes, there was severe congestion in the portal area with necrotic foci, enlargement of sinusoids, irregular hepatocytes, liver fatty degeneration (steatosis), aggregation and lymphocytes infiltration founds between hepatocytes, furthermore there were significant gradual reduction in space between portal area. These all the strong evidences for liver malfunctioning due to diabetes progression. This observation is in a line to other previous finding, Itohet *al.*^[36] have considered that fatty infiltration of liver as a precursor of cirrhosis in diabetic patients. Falchuket *al.*^[37] showed hepatic fatty steatosis and pericentral fibrosis in diabetic patients. Treatment with NS extract restored normal size and shape of the hepatocytes, reduces

sinusoidal space and reduces liver fatty degeneration. Similar protection of hepatic histopathological changes by NS was also reported in a previous study.^[38]

Present study demonstrated that decreased in blood glucose level and increased in body weight gain confirmed the hypoglycemic effect of NS. NS is potent hypolipidemic agent; this statement was supported by our findings. Our findings also showed the association between liver dysfunctioning and diabetic, in diabetes mice liver were distorted from their normal shape that induced to secrete liver marker enzymes, elevated level of this markers were predominant indicator for hepatic abnormalities. NS also ameliorated the effect of Diabetes on liver. Histological studies also confirmed the hepatoprotective effect of NS; NS restored normal size and shape of the hepatocytes.

Table 1: Effect of methanol extract of *N. Sativa* on body weight in experimental mice.

Blood weight Measuring days	Normal control	Diabetic control	Positive control	Treated mice (Dose)	
				50mg/kg body weight	200mg/kg body weight
Day-1	28.21±2.49	27.94±2.34	28.86±2.92	28.14±1.69	28.00±2.05
Day-8	29.17±3.06	26.73±2.68 ^b	27.01±1.23 ^b	26.72±2.27 ^b	27.81±2.21
Day-16	30.22±2.35	26.05±1.59 ^b	28.53±2.43 ^{bd}	26.54±2.53 ^b	28.13±2.68 ^d
Day-24	32.70±1.87	25.23±2.07 ^a	29.07±3.31 ^{bd}	28.64±2.07 ^{bd}	28.97±2.34 ^{bc}
Day-32	33.93±2.11	24.39±1.72 ^a	31.42±1.79 ^d	29.83±3.16 ^{bce}	30.02±2.67 ^{bc}

Body weights (in gm) were measure with 7 days interval for 32 days. Each value is the mean ± SEM (n=6). Body weight in the treated mice were significantly different from normal group at ^aP<0.001 and ^bP<0.05, whereas

^cP<0.001 and ^dp<0.05 indicated the difference from diabetic control group and ^eP<0.001 and ^fP<0.05 indicated the difference from positive control group.

Table 2: Effects of methanol extract of *Nigella sativa* on lipid profile of diabetic mice after 32 days treatment.

Groups	Result (mmol/L)					
	TG	TC	LDL-C	HDL-C	VLDL-C	
Normal control	2.05±0.16	5.44±0.06	4.03±0.28	1.09±0.12	0.52±0.03	
Diabetic control	3.67±0.22 ^a	8.61±0.26 ^a	7.19±0.20 ^a	0.93±0.06	0.69±0.06 ^b	
Positive control	2.13±0.23 ^c	5.76±0.09 ^{bc}	4.11±0.10 ^c	1.26±0.19 ^d	0.49±0.03 ^d	
Treated mice (Dose)	50mg/kg b.w.	2.70±0.19 ^{bct}	6.46±0.29 ^{act}	4.79±0.21 ^{bct}	0.98±0.14 ^t	0.58±0.06 ^d
	200mg/kg b.w	2.31±0.23 ^c	6.04±0.08 ^{bc}	4.53±0.14 ^{bct}	1.02±0.10 ^t	0.53±0.06 ^d

Each value is the mean ± SEM (n=6). Total Cholesterol, Triglycerides, LDL-C, VLDL-C and HDL-C in the treated mice were significantly different from normal control groups at ^aP<0.001 and ^bP<0.05; whereas ^cP<0.001 and ^dP<0.05 indicated significantly difference

from Diabetic control group with Positive control and Treated mice. At ^fP<0.05 indicated significantly difference from Positive control group with Treated mice.

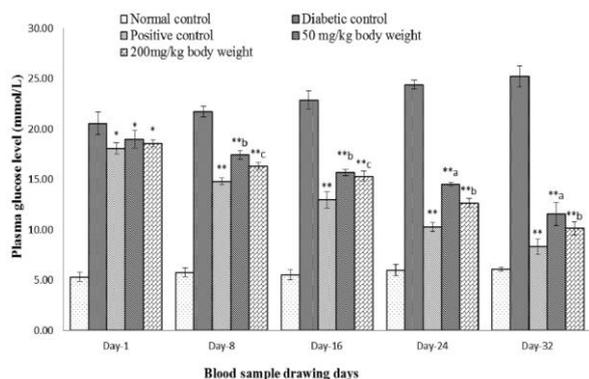


Figure 1: Effect of methanol extract of *N. Sativa* on blood glucose in alloxan-induced diabetic mice. Blood glucose levels were measured with 7 days interval for 32 days treatment. Each value is the mean \pm SEM (n=6). Blood glucose level in the treated mice were significantly different from normal group at ** P<0.001 and * P<0.05, whereas ^aP<0.001, ^bP<0.01 and ^dp<0.05 indicated the difference from positive control group.

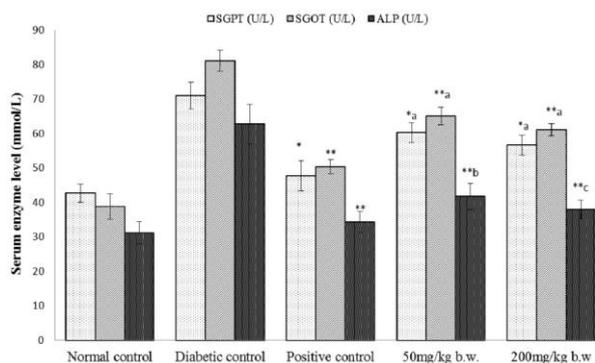


Figure 2: Effect of on liver function marker enzymes in alloxan induce diabetes mice. Each value is the mean \pm SEM (n=6). Serum SGPT, SGOT and ALP in the treated mice were significantly different from diabetes control groups at ** P<0.001 and * P<0.05; whereas ^aP<0.001, ^bP<0.01 and ^cP<0.05 indicated significantly difference from Positive control group with Treated mice.

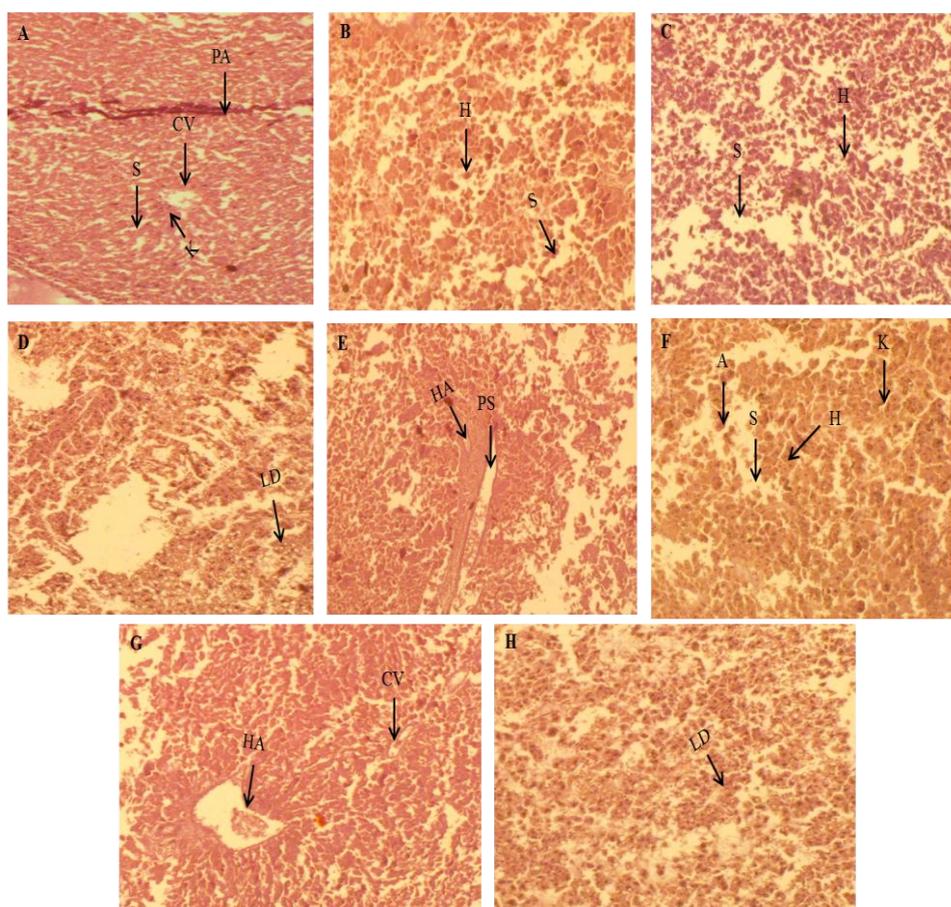


Figure 3: (A) and (B): histological pattern of liver from nondiabetic control mice (NC) showing normal-appearing of hepatocytes, portal area (PA), sinusoids (S), and Kupffer cells (K), central vein (CV) no fat accumulation (steatosis) is observed in control mice sacrificed at 32 days. (C), (D) and (E): liver from untreated diabetic rats sacrificed at 32 days showing enlarge sinusoids (s), irregular hepatocytes, large number of lipid droplets(LD), liver fatty degeneration (steatosis), shrinkage of portal space (PS). (F), (G) and (H): liver from NS treated diabetic mice sacrificed at 32 days showing large sinusoids (S), mild fatty degeneration, small lipid granules contain lipid droplets (LD), mild shrinkage of central vein (CV) and mild distortion of hepatocytes.

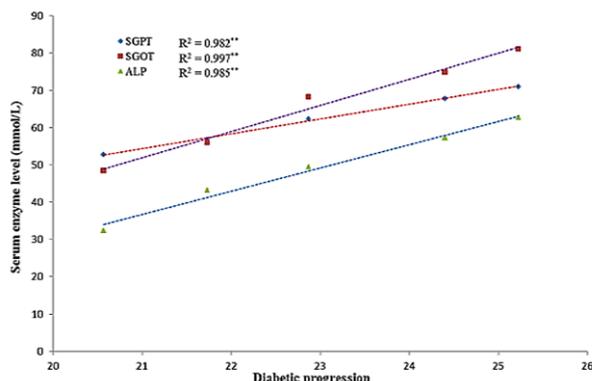


Figure 4: Correlation between serum level enzymes with diabetic progression. Where SGPT,SGOT and ALP were significantly correlated (P<0.01) with diabetic progression.**

CONCLUSION

In the present study, we found that methanol extract of NS has beneficial effect on FBG level and ameliorative effect on regeneration of hepatocytes. Therefore, NS has potential health benefit to be used as a pharmaceutical supplement in the management of diabetes. As NS is a very low cost herb, the potential cost benefit ratio will be in favor of benefit.

Declarations

Conflict of interest

The Authors declare that there is no conflict of interests.

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