ORIGINAL ARTICLE

HEPATORENAL CHANGES BY *NIGELLA SATIVA* SEEDS POWDER IN DIABETIC RATS

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Background: The rising rates of diabetes mellitus indicate a need for enhanced education and training in effectively preventing, screening, diagnosing and treating the condition. The objective of this study was to determine the possible beneficial effects of *Nigella sativa* (NS) seeds powder in treatment of diabetes mellitus. **Methods:** One hundred and forty adult male albino rats of local strain, aged 8 weeks, and weighing 150–200 g were chosen as an animal model for this study. The animals were divided into control and diabetic groups. Diabetes was induced by alloxan. After12 hour overnight fasting, morning blood samples were collected for determination of lipid profile, aspartate aminotransferase (AST), alanine amino transferase (ALT), creatinine and urea. **Results:** Lipid profile, AST, ALT and liver structure showed significant improvement with NS powder treatment. No statistically significant differences occur in creatinine or urea. **Conclusion:** NS seeds significantly decreased blood serum TC, TG and LDL, and increased HDL. Supplementations of NS reduced the ALT and AST levels. Oral administration of NS seeds did not produce any toxic effects on liver function evaluating hepatic enzymes level as well as histological picture of liver tissue.

Keywords: *Nigella stiva*, Diabetes mellitus, Liver, Kidney,

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INTRODUCTION

Complementary medicine has become popular worldwide over the past 20 years. Besides, the therapy based on chemotherapeutic agents in the present century has progressed towards naturopathy.[1] Plants have been the primary source of drugs, and many other currently available drugs have been directly or indirectly derived from plants. The anti-diabetic activity of herbs depends upon variety of mechanisms. Clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects such as bronchodilation, immunomodulation[2], antibacterial[3], hypotensive[4], anti-diabetic, hepatoprotective, gastroprotective, antihistaminic, anti-oxidative and neuroprotective[5].

The present study was designed to determine the possible beneficial effects of *Nigella sativa* (NS) powder in treatment of diabetes mellitus.

MATERIAL AND METHODS

One hundred and forty adult male albino rats of local strain, aged 8 weeks and weighing 150–200 g, were chosen as an animal model for this study. The animals were divided into two main groups:

Group (1): Normal rats (n=40) were divided into two equal subgroups: A=Control and B=Treated with NS powder.

Group (2): Diabetic group rats (n=100) were divided into 5 equal subgroups:

A. Control
B. Pre-treated with NS powder for 30 days before induction of diabetes mellitus
C. Pre-treated with NS powder for 30 days before induction of DM and insulin injection after induction of DM
D. Treated with NS powder only
E. Treated with both NS powder and insulin

The procedure was continued for 60 days.

Alloxan was used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic β-isletsby single injection of alloxa n monohydrate (65 mg/Kg) into the tail vein.[6] 50% dextrose-saline solution was administered subcutaneously within 12–24 hours after alloxan administration to minimize and prevent mortality.[7]

After12 hour overnight fasting, morning blood samples were collected from retro-orbital venous plexus before sacrificing. Blood was collected into a graduated glass centrifuge tube, and serum was separated by centrifugation at 5,000 rpm for 10 minutes. The separated serum was aliquotted and stored frozen in epindorffs' tube at -20 °C until used for the determination of lipid profile: Triglycerides (TG)[8], total cholesterol (TC), high density lipoprotein (HDL)[9], and low density lipoprotein (LDL)[10], aspartate aminotransferase (AST) and alanine amino transferase (ALT)[11], urea[12], and creatinine[13].

Data were analysed using SPSS-21. One way ANOVA was used for calculation of the descriptive statistics in studied groups, detection of any significant difference between groups and between different samples, performing multiple comparisons between each group and another, and each sample and another by

using the 'Post Hoc LSD' multiple comparison test; and $p<0.05$ was considered significant.

RESULTS

The Mean±SD of cholesterol levels in all groups at the end of experiment were 99.2±5.5, 93.46±5.01, 133.3±7.9, 114.7±3.5, 106.1±2.9, 121.5±4.6, and 111.2±4.09 mg/dl respectively. Comparing between groups showed significant differences. The Mean±SD of HDL levels in all groups at the end of experiment were 39.7±3.3, 45.3±2.4, 33.3±2.4, 34.7±1.8, 38.9±2.4, 30.7±2.7, and 37.6±3.0 mg/dl respectively. Comparison between groups showed significant differences. The Mean±SD of LDL levels in all groups at the end of experiment were 46.8±2.9, 43.1±2.6, 73.8±6.3, 54.2±3.9, 47.9±2.4, 51.8±4.1, and 50.9±3.8 mg/dl respectively. Comparison between groups showed significant differences. The Mean±SD of triglycerides level in all groups at the end of experiment were 91.7±4.9, 84.2±3.3, 113.7±6.1, 103.5±3, 100.1±3, 114.25±3.1, and 106.2±2.8 mg/dl respectively. There were significant differences between groups. (Figure-1).

The Mean±SD of AST at the end of experiment were 94.4±1.9, 84.4±1.9, 136.4±4.1, 104.4±1.9, 91.5±0.86, 114.4±1.9, and 122.9±2.4 U/ml. There were no statistically significant differences between groups 1-A and 1-B, but there were significant differences between 1-A & 2-A, 1-B & 2-B, 2B & 2C, 2-B & 2-D, 2-C & 2-E, 2-C & 2-D, and 2-A & 2-E. The Mean±SD of ALT at the end of experiment were 45.6±1.3, 40.3±1.9, 110.7±3.5, 48.5±1.1, 44.5±1.5, 82.06±4.1 and 62.06±5.3 U/ml. No statistically significant differences were seen between groups 1-A and 1-B, but there were significant differences between 1-A & 2-A, 1-B & 2-B, 2B & 2C, 2-B & 2-D, 2-C & 2-E, 2-C & 2-D, and 2-A & 2-E. (Figure-2).

Hepatocytes were arranged in trabeculae running radiantly from the central vein and were separated by sinusoids containing Kupffer cells. They were regular and contained large spheroidal nuclei with distinctly marked nucleoli and peripheral chromatin distributions. Some cells had two nuclei (Figure-3a).

Liver in diabetic control group revealed glycogen depletion in the hepatocytes, pyknosis of the nucleus and focal haemorrhages in some areas and areas of fat infiltration. There were marked vaculations of hepatocytes and congested blood vessels (Figure-3b).

In diabetic pre-treated with NS powder (for 30 day), the hepatocytes were arranged in trabeculae running radiantly from the central vein and were separated by sinusoids containing Kupffer cells. They were regular and contained large spheroidal nuclei with distinctly marked nucleoli and peripheral chromatin distribution. There was mild inflammatory infiltrate in portal tract (Figure-3c).

In diabetic treated with NS powder only after diabetic induction, the liver showed focal areas with minimal necrosis of the hepatocytes associated with mononuclear infiltration in portal tract (Figure-3d).

The Mean±SD of creatinine at the end of experiment were 0.6475±0.07, 0.6275±0.04, 2.05±0.27, 1.13±0.8, 1.04±0.18, 1.94±0.21, and 1.6±0.23 mg/dl. No statistically significant differences were seen between groups 1-A and 1-B, but there were significant differences between 1-A and 2-A, 1-B and 2-B, 2B and 2C, 2-B and 2-D, 2-C and 2-E, 2-C and 2-D, and 2-A and 2-E. The Mean±SD of urea at the end of experiment were 19.46±1.2, 17.3±0.9, 208.5±9.9, 52.8±5.05, 42.8±5.5, 91.4±6.4 and 80.1±4.1 mg/dl. No statistically significant differences were seen between groups 1-A and 1-B, but there were significant differences between 1-A & 2-A, 1-B & 2-B, 2B & 2C, 2-B & 2-D, 2-C & 2-E, 2-C & 2-D, and 2-A & 2-E. (Figure-4).

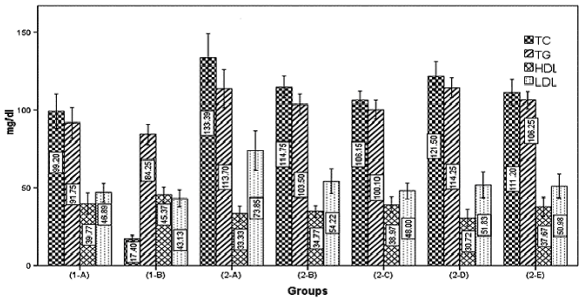

Figure-1: Effect of NS on lipid profile of adult male rats (Mean±SD)

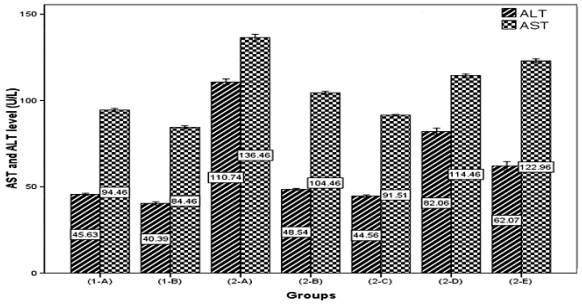

Figure-2: Effects of NS on AST and ALT level (U/L) of different groups (Mean±SD)

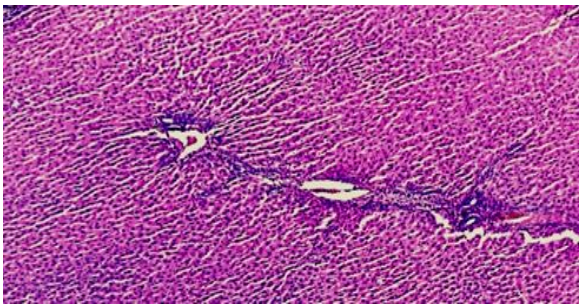

Figure-3a: Micrograph of liver in normal control group (H&E, ×400)

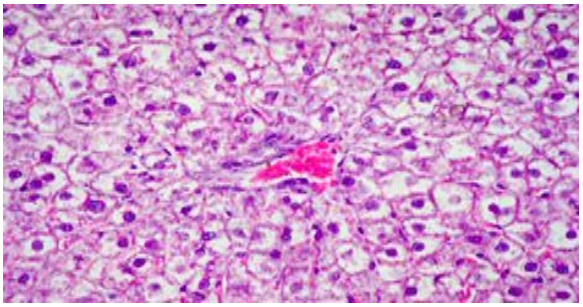

Figure-3b: Section in the liver of diabetic control group. Marked vaculation of hepatocytes and congested blood vessels. (H&E, ×400)

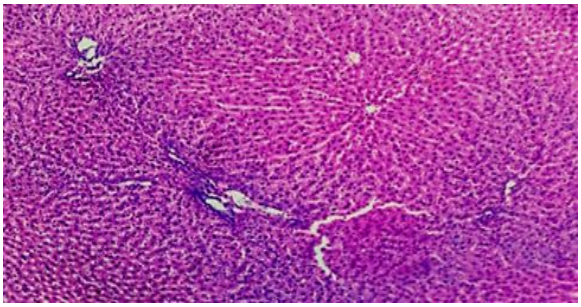

Figure-3c: Section in liver tissue of diabetic group pre-treated with NS powder for 30 days. (H&E, ×400)

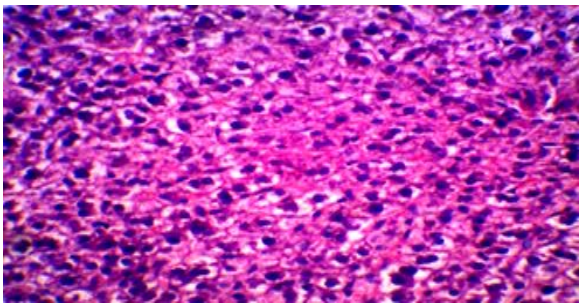

Figure-3d: Section in the liver tissue of diabetic group treated with NS. (H&E, ×400)

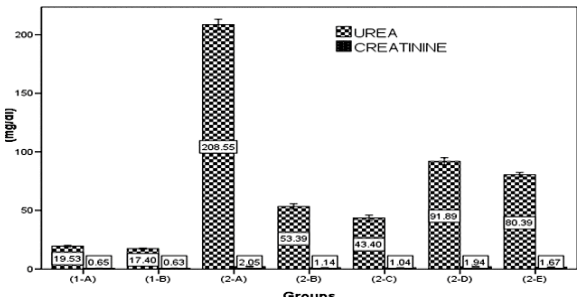

Figure-4: Creatinine and Urea levels (mg/dl) in all groups (Mean±SD)

DISCUSSION

It had been revealed that triglycerides (TG) accumulation increased considerably in diabetes mellitus. Hypercholesterolemia had been reported to occur in diabetics.[14] One of the interesting findings of our study was that NS seeds powder significantly decreased blood serum TC, TG and LDL, but increased HDL contents compared with control group. This is in agreement with Rahman *et al*[15] where they found that NS produces significant decrease in serum TC, TG, LDL levels and significantly increase in HDL level. Akhtar *et al*[16] also observed reduction in serum TG and TC contents, while serum HDL increases. Farzaneh *et al*[17] and Ibrahim *et al*[18] showed that supplementation with NS powder decreases TC, LDL, TG and increases HDL. Our findings were in line with the study of Shah *et al*[19] who indicated that NS seed decreases LDL and TG. On the other hand, Memon *et al*[20] concluded that NS seed only increases HDL and other lipid profile remains unchanged.

The possible anti-hyperlipidemic effects of NS can be attributed to its antioxidant components. NS can elevate total antioxidant capacity and antioxidant enzymes, decrease lipid peroxidation, and reduce free radicals directly and indirectly.[21–23] Antioxidant components can prevent lipid peroxidation and improve enzyme function which participates in lipid metabolism.[24] Unsaturated fatty acids such as linoleic and oleic acids, and polyphenol components in NS might participate in improving lipid profile parameters. Glycemic improvement can modulate lipid dysfunction particularly in patients with diabetes.[25] Other possible mechanisms may be due to reduction in insulin resistance and increasing in insulin sensitivity as NS, particularly its antioxidant components, can improve the intracellular pathways of insulin receptors and increase their sensitivity to insulin. Also, there is an association between losing weight and improvement in glucose status and lipid profile.

Zaoui *et al*[26] hypothesized that cholesterol lowering mechanism of NS seed oil is dependent on peroxisome proliferator-activated receptor (PPARα) activation. The mode of action of cholesterol reduction associated with consumption of fixed and essential oils of NS seed is multidimensional. The fixed oil of NS seed is rich in polyunsaturated fatty acids which mainly accounts for cholesterol lowering potential.[27]

The hypo-triglyceridemic effect of *Nigella sativa* is possibly due to its cholerectic activity as reported by Khan *et al*[28]. The cholerectic function of *Nigella sativa* is either by reducing the synthesis of cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine.[29]

DM produces degenerative changes in liver, probably due to increased lipid peroxidation.[30] It has been suggested that lipid peroxidation of biological membranes is often associated with the development of liver damage. It also has been suggested that the liver microsomes, particularly the xenobiotic transforming enzyme system, are very sensitive to lipid peroxidation. Thus, the oxidative decompositions of liver occurring

because of the increased lipid peroxidation could be the reason for the hepatocellular degeneration.[31]

Oral administration of NS seeds powder did not give any toxic effects on liver function evaluating hepatic enzymes level as well as histopathological changes of liver tissue. The supplementations of NS powder reduced the ALT and AST levels in treated rats compared to the control rats. Our findings agreed with Al Ameen and Musa[32], Dollah *et al*[33] and Gaur[34], who reported no toxic effects of NS on hepatic enzymes. Hepatoprotective effects of NS are due to some components, either thymoquinone and monoterpenes, or tocopherols, phytosterols, and phenols.[35]

The results of the present study showed that the supplementation of NS to the diets of rats for 8 weeks did not change the biochemical parameters of kidney function as well as histopathological investigations which illustrated normal architecture of kidney. No significant changes of serum urea and creatinine level occurred with group treated with NS. Our results agreed with Mathur *et al*[36] who found that NS has no significant changes on kidney functions. Zaouie *et al*[26], and Alghamdi[37] showed that there is no toxic effect of NS on kidney functions in mice. EL-Kholy *et al*[38] and AL Ameen and Musa[32] found that NS has a wide margin of safety on kidney.

CONCLUSION

Nigella stiva seeds significantly decreased blood serum TC, TG and LDL, and increased HDL. Supplementations of NS reduced the ALT and AST levels. Oral administration of NS seeds did not produce any toxic effects on liver function evaluating hepatic enzymes level as well as histological picture of liver tissue.

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