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CHANGES IN LIPID PROFILE, AST, ALT, UREA, AND CREATININE BY NIGELLA SATIVA SEEDS POWDER IN ADULT MALE ALBINO DIABETIC RATS

By

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ABSTRACT

Background: The rising rates of diabetes mellitus indicate a need for enhanced education and training in effectively preventing, screening, diagnosing and treating the condition. Objective: Determination of the possible beneficial effects of Nigella Sativa Seeds powder in treatment of diabetes mellitus. Material and Methods: One hundred and forty adult male albino rats of local strain, 8 weeks of age and weighing from 120 to 150 g, were chosen as an animal model for this study. The animals were divided into control and diabetic groups. Diabetes was induced by alloxan. The experiment continued for 60 days of Nigella Sativa seeds powder treatment. After12 hours over night fasting at the end of experiment, morning blood samples and liver specimens were collected for the determination of lipid profile, aspartate aminotransferase "AST", alanine amino transferase "ALT", creatinine, urea and liver architecture. Results: Lipid profile, AST, ALT and liver structure showed significant improvement with NS powder treatment. No statistically significant differences occur in creatinine or urea. It caused a sort of protection to the hepatocytes from the effect of diabetes mellitus. Conclusion: Oral administration of NS seeds did not give any toxic effects on liver function evaluating hepatic enzymes level as well as histopathological changes of liver tissue.

INTRODUCTION

Complementary medicine has become popular worldwide over the past 20 years. Besides, the therapy based on chemotherapeutic agents in the present century towards naturopathy progressed (Chauhan et al., 2010). Plants have been the primary source of drugs, and many other currently available drugs have been directly or indirectly derived from plants. The antidiabetic 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 (Kanter, 2009), antibacterial (Gali-Muhtasib et al., 2006), hypotensive (Butt and Sultan, 2010), antidiabetic, hepatoprotective, gastroprotective, antihistaminic, antioxidative and neuroprotective (Kanter et al., 2009).

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, 8 weeks of age and weighing from 120 to 150 g, were

chosen as an animal model for this study. They were kept in suitable cages (20x32x20 cm for every 4 rats) at room temperature with the natural light-dark cycle. They were maintained on a standard diet of commercial rat chow and tap water in the department of Physiology, Al-Azhar Faculty of Medicine. They were kept for 10 days for the adaptation to the new environment before the start of the experiment. The animals were divided into two main groups:

- **A. Group (1):** Normal rats (40 rats) were divided into two equal subgroups:
- 1. Control. 2. Treated with NS powder.
- **B.** Group (2): Diabetic group rats (100 rats) were divided into 5 equal subgroups:
- 1. Control.
- 2. Pretreated with NS powder for 30 days before induction of diabetes mellitus.
- 3. Pretreated with NS powder for 30 days before induction of DM and insulin injection after induction of DM.
- 4. Treated with NS powder only.
- 5. Treated with both NS powder and insulin.

The procedure continued for 60 days.

• Induction of diabetes: Alloxan has been used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic β-isletsby single injection of alloxan monohydrate (65 mg/kg) into the tail vein (Rohilla and Ali. 2012). dextrose-saline solution was administered subcutaneously within 12-24 h after alloxan administration to minimize and prevent mortality (Federiuk et al., 2004).

After12 hours over night fasting, morning blood samples were collected from retro-orbital venous plexus sacrification. before Blood collected into a graduated glass centrifuge tube, and serum was separated by centrifugation at 5000 r.p.m 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" (Bucolo and David, **1973**), total cholesterol "TC", high density lipoprotein "HDL" (Friedewaled et al., 1972), low density lipoprotein "LDL" (Ellefson and Caraway. **1976**). aspartate aminotransferase "AST. alanine amino transferase "ALT" (Miura, 1999), urea (Patton and Crouch, 1977), and creatinine (Young, 1955).

Statistical analysis: One way Analysis Of Variance (ANOVA) test was used for calculation of the descriptive statistics in studied groups (means + standard deviations), detection of any significant difference between different 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. P<0.05 was considered significant. The computer program SPSS version "21" was used.

RESULTS

Lipid profile (Figure 1):

The means \pm SD of cholesterol level 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 between groups (1-A &1-B), (1-A &2-A), 1-B & 2-B), (2-B & 2-C), (2-B &2-D), (2-C & 2-E), (2-C & 2-D), and (2-A & 2-E).

The means \pm SD of HDL level 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. Comparing between groups showed significant differences between groups (1-A & 1-B), (1-A &2-A), (1-B &2-B), (2-B & 2-C), (2-B &2-D), (2-C &2-E), (2-C &2-D), and (2-A &2-E).

The means \pm standard deviations of LDL level 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. Comparing between groups showed significant differences between groups (1-A &1-B), (1-A & 2-A), 1-B & 2-B), (2-B &2-C), (2-B &2-D), (2-C &2-E), (2-C &2-D) and (2-A & 2-E).

The means \pm SD of triglycerides level in all groups at the end of experiment were 91.7 \pm 4.9, 84.2 \pm 3.3, 113.7 \pm 6.1, 103.5 \pm 3. 100.1 \pm 3, 114.25 \pm 3.1, and 106.2 \pm 2.8 mg/dl respectively. Comparing between groups showed significant differences between groups (1-A & 1-B), (1-A & 2-A), 1-B & 2-B), (2-B &2-C), (2-B and 2-D), (2-C & 2-E), (2-C &2-D), and (2-A & 2-E).

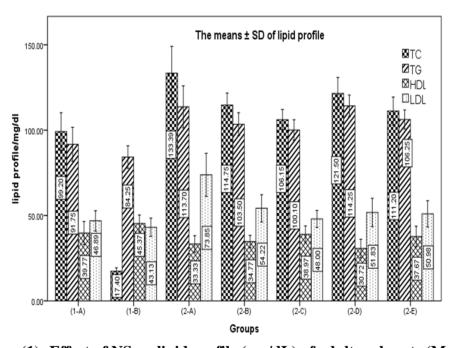


Figure (1): Effect of NS on lipid profile (mg/dL) of adult male rats (Mean±SD).

Effect of NS on AST and ALT level (Figure 2):

The means \pm standard deviations of AST at the end of experiment were 94.4 \pm

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. No statically significant differences between groups (1-A and 1-B), but there were significant differences between (1-A

&2-A: P), 1-B & 2-B), (2B &2C), (2-B &2-D), (2-C & 2-E, (2-C & 2-D) and (2-A & 2-E).

The means \pm standard deviations of ALT at the end of experiment were 45.6 \pm 1.3, 40.3 \pm 1.9, 110.7 \pm 3.5, 48.5 \pm 1.1, 44.5 \pm 1.5, 82.06 \pm 4.1 and 62.06 \pm 5.3

U/ml. No statistically significant differences between groups (1-A and 1-B), but there were significant differences between (1-A & 2-A: P), 1-B &2-B), (2B & 2C), (2-B & 2-D), (2-C &2-E, (2-C&2-D) and (2-A &2-E).

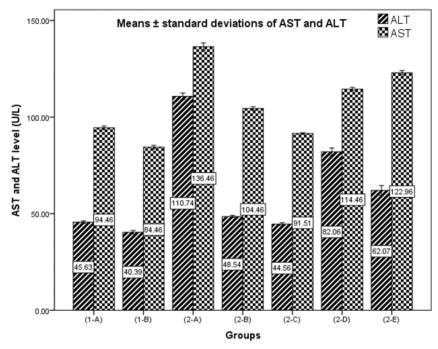


Figure (2): Effects of NS on AST and ALT level (U/L)of different groups (Mean±SD)

Histopathological results of the liver (Figs. 3):

Hepatocytes were arranged in trabeculae running radially from the central vein and were separated by sinusoids containing Kupffer cells. They were regular and contained a large spheroidal nuclei with a distinctly marked nucleoli and peripheral chromatin distributions. Some cells have two nuclei (Fig. 3-a). Liver in diabetic control group revealed glycogen depletion in the hepatocytes, pyknosis of the nucleus and focal hemorrhages in some areas and areas of fat infiltration. There were marked vacuolations of hepatocytes

and congested blood vessels (Fig.3-b). In diabetic pretreated with NS powder (for 30 day), the hepatocytes were arranged in trabeculae running radially from the central vein and were separated by sinusoids containing Kupffer cells. They were regular and contained a large spheroidal nuclei with distinctly marked nucleoli peripheral chromatin and distribution. There was mild inflammatory infiltrate in portal tract (Fig.3-c).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 (Fig. 3-d).

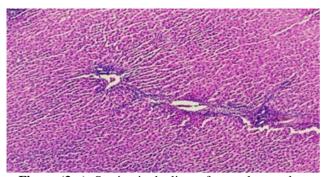


Figure (3-a): Section in the liver of normal control group (Hx&E, 400X).

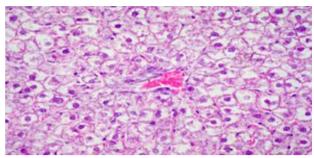


Figure (3-b): Section in the liver of diabetic control group (Hx&E, 400X): A: Marked vaculations of hepatocytes and B: Congested blood vessels (Hx&E, 400X)

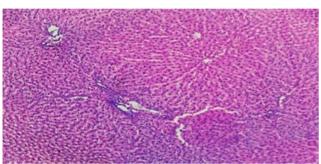


Figure (3-c): Section in the liver tissue of diabetic group pretreated with NS powder for 30 day (Hx&E, 400X).

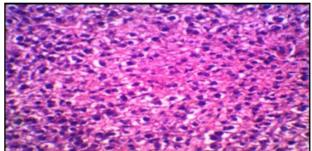


Figure (3-3): Section in the liver tissue of diabetic group treated with NS (Hx&E, 400X).

Figure (3): Histopathological changes in liver.

Effects of NS on kidney function of adult male rats (Figure 4):

The means \pm standard deviations 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 difference 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-B)

C and 2-E, (2-C and 2-D) and (2-A and 2-E).

The means \pm standard deviations 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 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) & (2-A & 2-E).

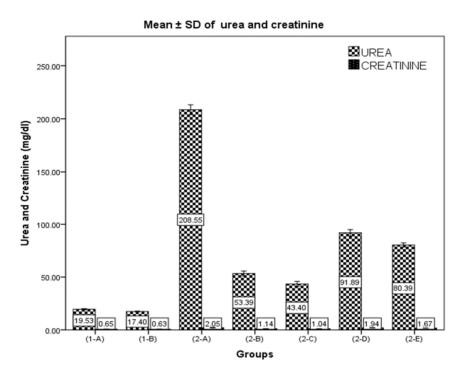


Figure (4): Creatinine and Urea levels (mg/dl) in all groups (Mean± SD)

DISCUSSION

It had been revealed that triglycerides (TG) accumulation increased considerably in diabetes mellitus. Hypercholesterolemia had been reported to occur in diabetics (Islam et al., 2012). 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 agreed with Rahman et al. (2015)where they found that NS produces significant decrease in serum TC, TG, LDL levels and significantly increase in HDL level. Akhtar et al. (2003) also observed reduction in serum TG and TC contents, while serum HDL increases. Farzaneh et al. (2014) and Ibrahim et al. (2014) 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.** (2012) who indicated that NS seed decreases LDL and TG. **Memon et al.** (2012) concluded that NS seed only increases HDL and other lipid profile remains unchanged.

possible anti-hyperlipidemic The 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 (Kaleem et al., 2006, Abdelmeguid et al., 2010; and Sultan et al., 2014). Antioxidant components can prevent lipid peroxidation and improve enzyme function which participates in lipid (Yin 2010). metabolism al., Unsaturated fatty acids such as linoleic oleic acids, and pholyphenol components in NS might participate in

improving lipid profile parameters. Glycemic improvement can modulate lipid dysfunction particularly in patients with diabetes (Kuenen et al., 2011). Other possible mechanisms may be due to reduction in insulin resistance 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. (2002) hypothesized that cholesterol lowering mechanism of N. sativa seed oil is dependent on peroxisome proliferator-activated receptor (PPAR α) 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(**Hossain et al., 2007**).

The hypo-triglyceridemic effect of Nigella sativa is possibly due to its cholerectic activity as reported by **Khan et al.** (2013). 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 (**El-Bahr**, 2007).

DM produces degenerative changes in liver, probably due to increased lipid peroxidation(Meral et al., 2001). 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 (Noureddin et al., 2015).

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 intreated rats compared to the control rats.Our findings agreed with Al Ameen and Musa (2011), Dollah et al.(2013) and Gaur (2015) who reported no toxic effects of NS on hepatic enzymes. Hepatoprotective effects of NS are due to some components such as either thymoquinone and monoterpenes, or tocopherols, phytosterols, and phenols (Asadi-Samani et al., 2015).

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 kidnev 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. (2011) whostated that NS has no significant changes on kidney functions. Zaouie et al. (2002) and Alghamdi (2003) showed that there is no toxic effect of NS on kidney functions in mice. EL-Kholy et al. (2009) and AL Ameen and Musa (2011) found that NS has a wide margin of safety on kidney.

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أقسام الفسيولوجيا والباثولوجيا *- كليات الطب _ جامعتى الأزهر وسوهاج **

خلفية البحث: يدفع تزايد الإصابة بمرض الداء السكرى إلى الحاجة للتثقيف والتدريب لإحداث تأثيرات فعالة لمنع ومراقبة وتشخيص وعلاج حالاته.

الهدف من البحث: تقدير التأثيرات النافعة الممكنة لمسحوق بذور الحبة السوداء في علاج مرض الداء السكري.

مواد وطرق البحث: أجرى البحث على 140 جرذا أبيضا بالغا من سلالة محلية أعمارها ثمانية أسابيع وتتراوح أوزانها بين 120 – 150 جم وتم إحداث مرض الداء السكرى بالألوكزان. واستمرت التجربة لمدة ستين يوما تم خلالها العلاج بمسحوق بذور الحبة السوداء. وقد أخذت عينات من الدم وعينات من الكبد في نهاية مدة التجربة لفحص نمط الدهون ووظائف الكبد والكلي.

النتائج: حدث تحسناً ذو دلالة إحصائية في نمط الدهون ووظائف الكبد بتأثير مسحوق بذور حبة البركة، كذلك حدث تحسنا في وظائف الكبد إلا أنه كان ذا دلالة غير إحصائية، كما حدث نوع من الحماية لخلايا الكبد من تأثير مرض البوال السكري.

الإستنتاج: لم يحدث مسحوق بذور الحبة السوداء أي تأثير ضار لخلايا الكبد.