The Egyptian Journal of Hospital Medicine (October 2013) Vol. 53, Page 1072– 1082

Protective Effect of Nigella Sativa Against Diabetic Complications on The Liver in White Male Rats

Nassar Ayoub Abdellatif
Department of Anatomy, Faculty of Clinical Pharmacy, Taif University, Saudi Arabia
andFaculty of medicine, Sohag University, Egypt.
Corresponding author: Nassar Ayoub Abdelatif Omar,00966508772881. Zip code: 2197 E-mail address: nassarayoub@yahoo.com.

Abstract

Aim of the work: The effect of diabetes on the liver is associated with histological changes. The aim of the present study was to evaluate the histological changes following administration of nigella sativa (NS) in the streptozotocin (STZ) induced diabetes mellitus in rats.

Materials and Methods: Thirty six male white rats (n=36), weighing (180–230 g) were taken for this study. The animals were divided into 3 groups: 1- Normal control group. 2- Diabetic group. 3- Diabetic group treated by Nigella Sativa (N.S) oil. Diabetes was induced in the experimental rats via intraperitoneal injection of streptozotocin (45 mg/kg body weight) in a single dose. The fasting blood glucose was estimated, 5ml\kg of body weight of N. sativa oil was given orally for three weeks. After an overnight fast, the animals were sacrificed. The livers were identified, weighed and observed for any gross appearance and color changes and tissues were preserved for histopathological studies using hematoxylin & eosin (H&E) and periodic acid Schiff (PAS) stains.

Results: In streptozotocin treated animals, the body weight was significantly decreased compared to normal rats, while treating diabetic rats with *N. sativa* oil showed significant increases in the body weight. Administration of nigella sativa oil to diabetic rats resulted in a significant decrease in blood glucose after three weeks compared to untreated diabetic rats. In untreated diabetic group, there was a significant decrease in the liver glycogen. Light microscopic examination of the liver of diabetic rats revealed profound histological changes. Nigella sativa consumption could reverse most of these histological and biochemical changes in the liver of the diabetic group owing to its hypoglycemic and antioxidant effect.

Conclusion: The Nigella Sativa due to its antioxidant role may be helpful in reversing the changes in the liver in diabetes mellitus.

Keywords: *Liver, Diabetes mellitus, Streptozotocin, Streptozotocin diabetes, Nigella Sativa.*

Introduction

Streptozotocin (STZ): Diabetes mellitus (DM) is one of the most common lifestyle diseases. Type 2 diabetes had global prevalence estimate of 2.8% in the year 2000 and is projected to be 4.4% in 2030 [1]. Prevention and control of DM is a major challenge and requires molding lifestyle towards more physical activity and less calorie intake avoiding sedentary habits. However most people find it difficult to change their lifestyle and look for a less cumbersome alternative. A traditional component of food that can reduce appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, body weight, and can stimulate glucose induced secretion of insulin from ß-cells in pancreas, may prove to be useful for prevention and control of diabetes mellitus. Most of these actions have been shown by seeds of nigella sativa and their constituents in animal experiments and at the same time have not exhibited adverse effects [2].

Streptozotocin (STZ) is a naturally occurring nitrosourea with molecular weight of 265 and empirical formula of C14 H27 N5 O12 [3]. It is widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells [4&5]. The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin [6]. The effects of STZ on

DOI: 10.12816/0001669

different organs have been extensively studied. STZ has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis [7]. STZ is a nitrosourea compound which generally shares similar fate of disposition with other nitrosoureas and is a drug of choice in islet cell carcinoma and malignant carcinoid tumors. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration [8&9]. STZ given intravenously or intraperitoneally to laboratory mice in multiple sub-diabetogenic doses induces pronounced pancreatic insulitis with eventual destruction of insulin secreting beta cells and diabetes mellitus. In an experimental study in rats, streptozotocin given intraperitoneally in a dose of 45 mg/kg body weight of animals, effectively produced hyperglycaemia [4&5]. In another study in rats, STZ injected in a dose of 65 mg/kg body weight effectively produced hyperglycemia and gastric mucosal ulcerations [8&9]. The incidence and severity of lesions produced by STZ in pancreas, liver, kidney and GIT, progressively increased with time from one to six weeks post treatment [9]. Studies have shown an association between specific diabetic complications and liver enzyme alterations [10], but only limited data is available on the possible association between diabetic nephropathy and kidney enzyme alterations [11].

NIGELLA SATIVA: N. sativa is an annual herbaceous plant that belongs to the family (Ranunculaceae). It contains both fixed and essential oils, proteins, alkaloids and saponin. Thymoquinone, the major component of the essential oil, is the biologically active gradient of this plant [12]. It was reported that, thymoquinone (TQ) has many medicinal properties like; anti-oxidant [13, 14 &15], anti-inflammatory [16], anti-tumor [17], analgesic and other properties [18&19]. On the other side, Kapoor [2] proved that TQ protects hepatic tissue from deleterious effects of toxic metals such as lead, and attenuates hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride.

Material and Methods

Materials: Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Al-Saggaf Est. (Jeddah, Saudi Arabia). N. sativa oil was obtained from Dreams Essential Oils Est. (Jeddah, Saudi Arabia). N. sativa oil was extracted by steam distillation. The major compounds of N. sativa oil were thymoquinone (29.7%), pcymene (23%), carvacrol (11.5%), α-pinene (8.6%), 4-terpineol (3.7%), longifoline (2.8%), carvone (1.8%)
and t-anethole (0.8%) [20].

Experimental animals: Male white rats weighing (180–230 g) were obtained from The Animal Experimental Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. The rats were housed in well-aerated individual cages in an animal room and maintained in a temperature-controlled room (24 ± 1 °C) with a 12 h light/12 h dark cycle, 55±10 % humidity. They were fed with normal commercial chow and water ad libitum. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals and all experimental procedures were approved by the Animal Care and Use Committee of King Abdul Aziz University.

Induction of diabetes: The experimental animals were fasted for 12 hours and then diabetes was induced by a single intraperitonial injection of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA), dissolved in a freshly prepared physiological saline solution (0.9% NaCl) at a dose
of 45 mg/kg body weight, while normal control rats received only the saline solution (0.9% NaCl) in the same volume and through the same route. After injection, all animals were returned to their cages and given free access to food and water. After 3 days, the fasting blood glucose levels were measured from tail blood samples by using an OneTouch Ultra® glucometer (Lifescan; Johnson &

Johnson, Milpitas, CA, USA). Animals with blood glucose levels more than 140 mg/dl were considered diabetic and used for the experiment. N. sativa seeds (black seed) were cleaned air-dried and then were powdered mechanically to prepare a suspension in isotonic saline solution. The suspension (1.25mg powder of N. sativa + 100 ml isotonic saline) was freshly prepared and left a few minutes before administration.
Streptozotocin (STZ) was dissolved in 10 ml sodium citrate buffer, pH 4-4.5, made isotonic by the addition of an appropriate volume of 0.25 ml NaCl [21].

Experimental design: Thirty six adult male white rats weighing (180–230 g) were housed in metabolic cages on a 12-h light/dark cycle at a temperature of 22-24°C [22]. The animals were divided into three groups;
1- Group I: Normal control (Non-diabetic normal rats) received normal commercial chow and water ad libitum.
2- Group II: STZ-Control (Diabetic control_rats) received the same diet given in group I.
3- Group III: STZ + N. sativa oil 5ml\kg of body weight. All of the experimental groups received the treatments for a period of three weeks. After an overnight fast, the animals were sacrificed at the end of third week. The livers were extracted, weighed, identified and observed for any gross appearance and color change with magnifying glass and tissues were preserved for histopathological studies. For light microscopic examination, the liver tissue was fixed in 10 % buffered formaline and embedded in paraffin. Five um sections were cut and stained with Hematoxylin and Eosin and Periodic Acid Schiff (diastase test was performed) [23]. A half gram of liver tissue from each animal was used for glycogen estimation in the liver [24].
Body weight: Rats were weighed at the start of the experimental period and weekly for 3 weeks using a digital balance. These weights were determined at the same time during the morning. The data were analyzed using a computer program for Student t-test. All the results were expressed as mean ± S.D. Difference between groups were considered significant when $p < 0.05$.

Results

Body weight changes (Table 1, Charts1, 2&3): There was no death of animal in the experimental period. Table1 shows mean body weight of both control and experimental groups after one week, two weeks and three weeks. There were significant effects for the treatment ($p<0.001$) and duration ($p<0.001$). The treatment x duration ($p<0.001$) interaction was also significant. It is obvious that control animals showed a progressive increase in body weight with the lapse of time. STZ-induced diabetic rats had the lowest body weight change after three weeks ($p<0.001$). STZ-induced diabetic rats given diets containing N. sativa oil showed marked improvement in their body weight in comparison with STZ-induced diabetic group ($p<0.05$).

The gross examination showed that, the liver is mildly enlarged with a rounded inferior margin, smooth capsule and a pale yellow brown greasy cut surface. The parenchyma is pale yellow color. The gross appearance of the liver improved upon treatment with N.S oil, the liver decreased in size and appeared brighter and less greasy. These changes were partly observed by quantifying the lipid droplets by LM (Fig. 2). Therefore, it could be concluded that N.S improved steatosis of the liver in type 2 diabetic rats. There was no obvious fibrosis in these images, suggesting that the diabetic rats in our study only suffered from fatty liver and had not yet developed cirrhosis.
Blood glucose (Table 2, Charts 4):: The mean values of blood glucose of both control and experimental groups are presented in table 2 and chart 5. STZ-induced diabetic rats showed a highly significant ($p<0.001$) increase in the levels of blood glucose, registering increases of 155% after three weeks compared to the controls. Administration of *N. sativa* oil to diabetic rats resulted in a significant ($p<0.001$) decrease in blood glucose levels of 15%, after three weeks, compared to untreated diabetic rats.

As regard to the liver histology: Liver histology in group I (control group) showed normal histological appearance of the liver tissue, which consists of a vast interanastomosing network of hepatocytes arranged in single-cell thick plates separated by vascular sinusoids. The hepatocytes along with vascular channels form organized micro structures which serve as structural and functional units (Fig.1). Where as those of group II (Diabetic rats) showed that intra-cytoplasmic accumulation of triglyceride (neutral fats). Fat droplets displace the centrally located nucleus forming fate vacuoles. The vacuoles pushing the nucleus to the periphery of the cell giving characteristic signet ring appearance (macrovesicular fatty change). These vesicles are well delineated and optically "empty". Many hepatocytes possessed shrunken nuclei, ambiguous cell boundaries, granulated cytoplasm and dilated sinusoids (Fig.2). The liver histological examination of diabetic rats (Group III) received N.S showed that most hepatocytes had normal sized nuclei, the number and the size of vacuoles were markedly decreased, the cell boundaries were clear and the size of sinusoids were normal (Fig.3).

The most of the hepatocytes of group I are studded with PAS positive granules (Fig.4, Table 2 and Chart 4), but the liver specimens obtained from STZ treated animals (Group II) showed significant decrease by about 30% ($p < 0.05$) in the hepatic content of glycogen as compared to that of the control. Glycogen deposition was sparse and irregular; morphology of hepatocyte was also altered by the presence of the fatty globules (Fig. 5. Table 2 and Chart 4), while in group III the hepatic glycogen content was significantly increased ($p<0.01$) by about 21% in comparison to that of the group II (Fig. 6, Table 2 and Chart 4).

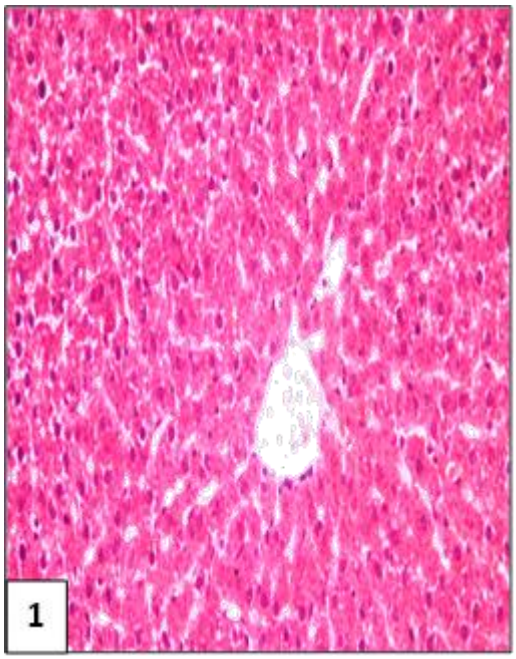


Fig. (1): Section in liver tissue (Group I) showing normal hepatic parenchymal (H& E X200).

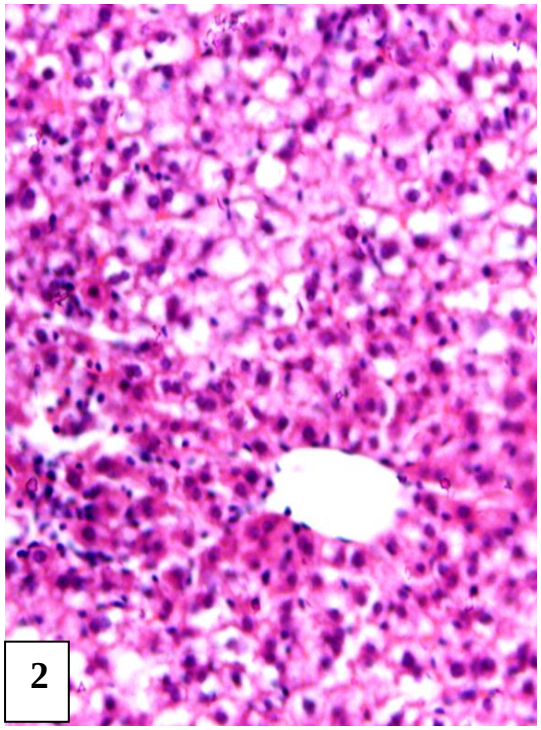


Fig. (2): Section in liver tissue in streptozotocin treated rat (Group II) showing marked steatosis. Numerous large fat cells were seen in the cytoplasm of numerous hepatocytes. Morphology of hepatocyte was also altered by the presence of the fatty globules. (H& E X200).

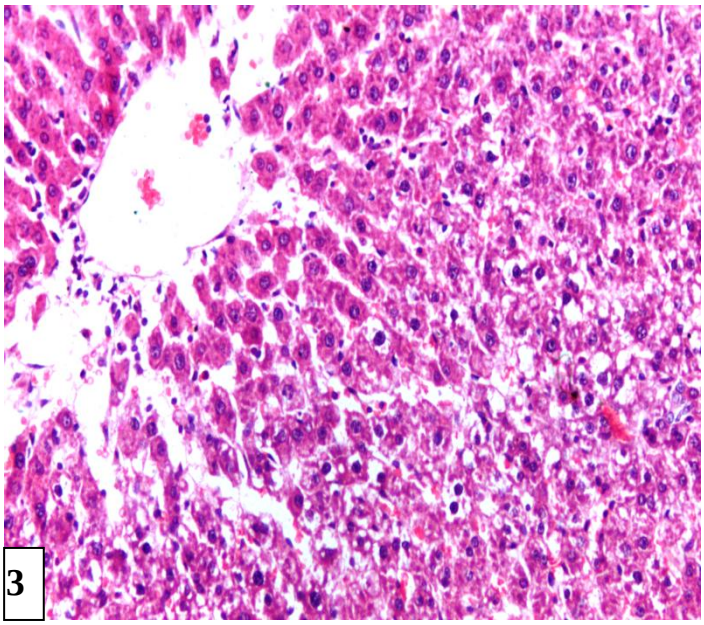


Fig. (3): Section of streptozotocin + N.S treated rat (Group III) showed that most hepatocytes had normal sized nuclei, the number and the size of vacuoles were markedly decreased, the granulated appearance of the cytoplasm was retained, the cell boundaries were clear and the size of sinusoids were normal (H& E X200).

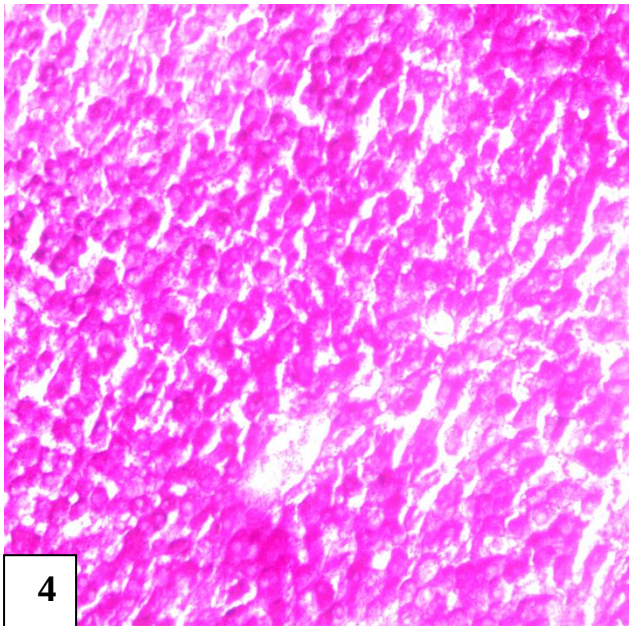


Fig. (4): Photomicrograph of group I showing that most of the hepatocytes are studded with PAS positive granules. (Control group, PAS X 200).

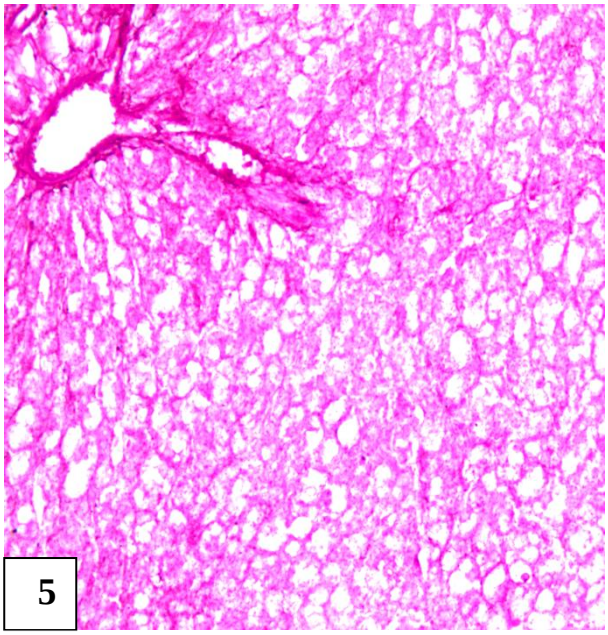


Fig. (5): Photomicrograph of group II showing few PAS positive granules in the hepatocytes. (STZ treated group, PAS X 200).

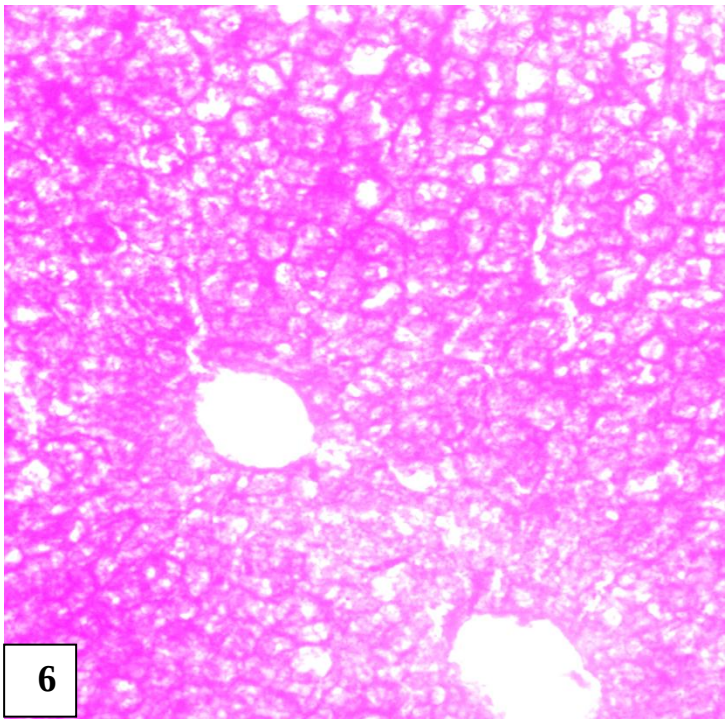


Fig. (6): Photomicrograph of group III showing PAS positive granules in most of hepatocytes (STZ + N.S treated group, PAS X 200).

Table 1: Effects of N. sativa oil supplementation on body weight in STZ- diabetic rats.

Treatment / Group	Week 0	week 1	Week2	week 3
Group I. (Normal control)	200.11±2.3	215.80 ± 3.2 (15.69±2.3) ***	222.33 ± 2.8 (6.48±2.3) **	263.33 ± 3.4 (41±1.5) ***
Group II (STZ)	199.21±1.55	180.43 ± 1.9 (-18.78±2.3) ***	178.22 ± 2.1 (-2.21±3.5) **	175.60 ± 2.1 (-2.62±4.1) **
Group III (STZ + N. sativa oil)	201.22±1.82	193.25 ± 2.3 (-7.97±2.3) **	188.38 ± 2.4 (-4.87±3.6) ***	204.70 ± 2.2 (16.32±3.6) *** ##

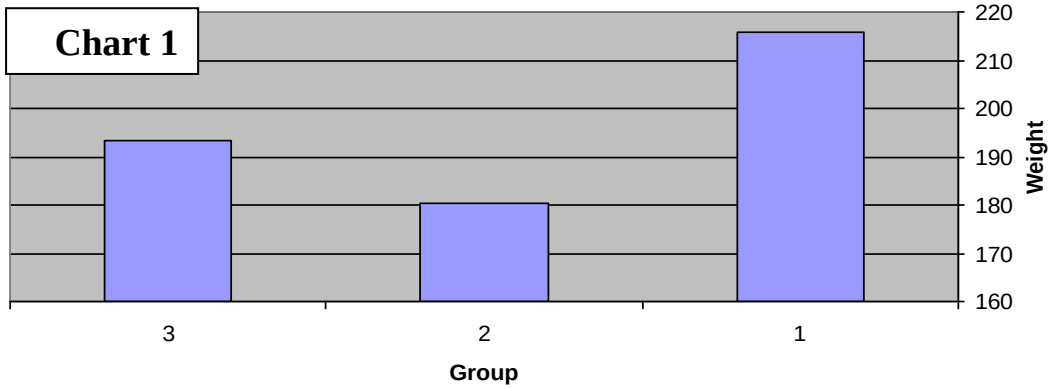
The number of animals was 12 for each group. Percent changes are included in parentheses. All values are expressed as means ± SE. (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$) when compared to control values. (##$p < 0.01$) when compared to STZ values.

Table 2: Shows liver glycogen and blood glucose in the three different groups.

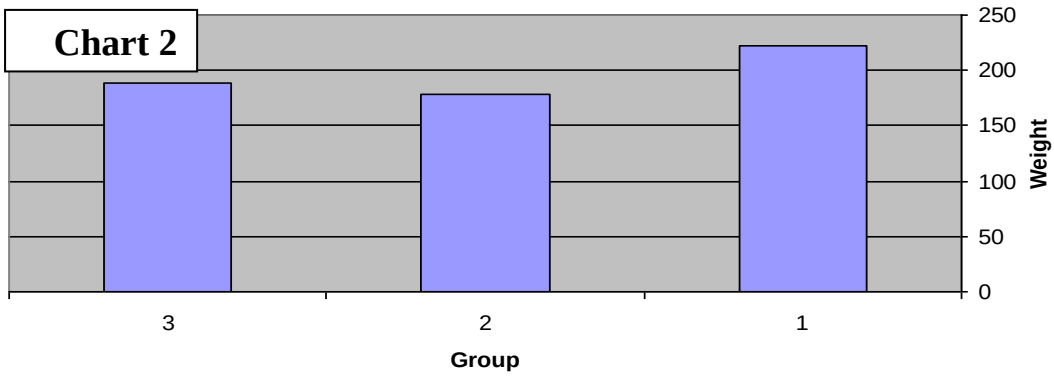
Group	Group I	Group II	Group III
Parameters	Control	STZ treated	STZ + N.S
Liver glycogen mg⁄g tissue% of change	8.3 ±1.45	5.3 ± 1.14	7.4 ± 1.12
Glucose (mg/dl)	95.12 ± 2.3	277.32 ± 3.4	237.18 ± 2.8***

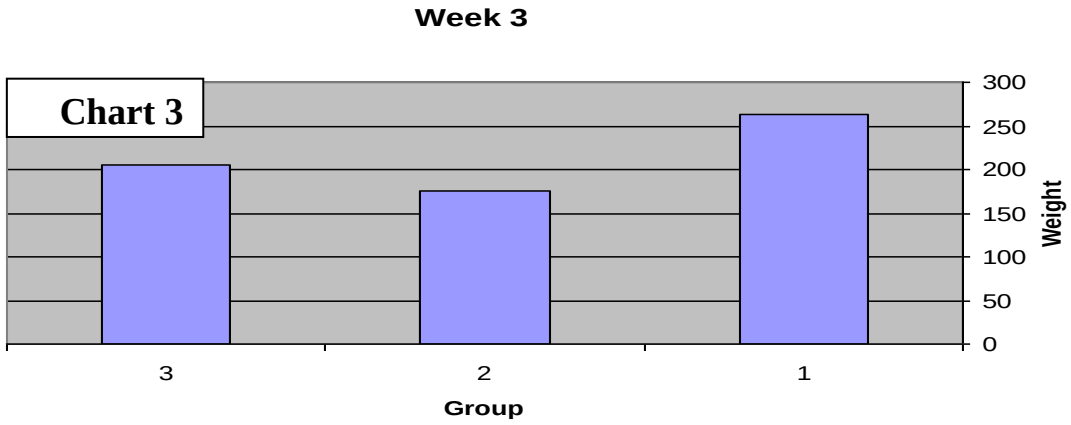
The number of animals was 12 for each treatment. All values are expressed as means ± SE. Significantly different from untreated STZ-induced diabetic rats (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Week 1

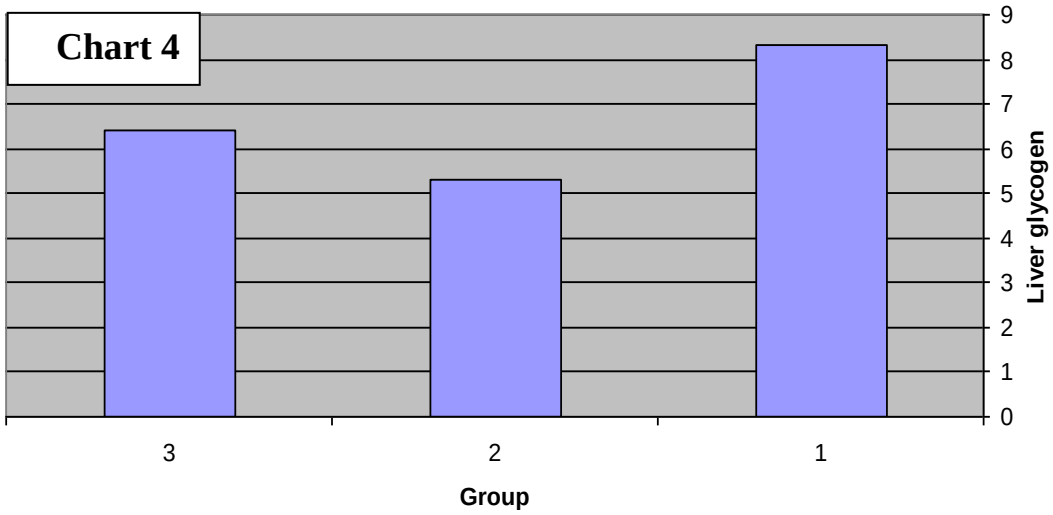


Week 2





Charts 1, 2 &3: Showing the correlation between the body weights in the three animal groups.



Charts 4: Showing the correlation between the liver glycogen in the three animal groups.

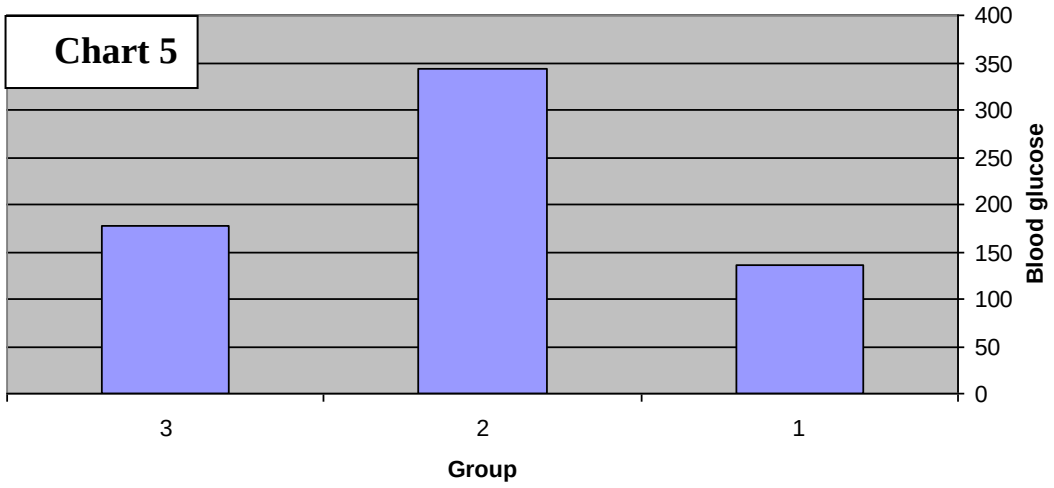


Chart 5: Showing the correlation between the blood glucose in the three animal groups.

Discussion

Results of the present study showed that diabetic rats exhibited a significant increase in blood glucose level. This result is in consistent with the finding of *Augusti and Sheela* [25] and *Campos et al.,* [26] in rats, *Kumar and Reddy* [27] in mice and *Jain and Vyas* [28] in rabbits. Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in STZ-induced diabetes mellitus rats [29, 30 & 31]. In the present study, the oils of *N. sativa* significantly reduces blood glucose levels in STZ-induced diabetic rats after 3 weeks of treatment, which also demonstrates that there is significantly higher rate of glucose disposal. Similar observations were also obtained by *Al-Awadi et al.* [32], who reported that plant mixture extract comprising of *N. sativa*, Myrrh, Gum Arabic, Gum Asafoetida and Aloe to have a blood glucose lowering effect. Also, the intraperitoneal administration of volatile oil of *N. sativa* to fasting normal and alloxandiabetic rabbits produced significant hypoglycemic effects [33]. However, the mechanism of this oil used has not been clearly defined. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent streptozotocin [34]. Previous studies demonstrated that the essential oil of black seed and its active constituents has proven free radical scavenging and antioxidant activities [35, 36 &37]. Based on above mentioned reports, we suggest that the possible mechanism of action by the oil of *N. sativa* could be related to antioxidants that aid to recover from impaired metabolism of glucose. In the present study, STZ-induced diabetic rats given the control diet had the lowest body weight change after three weeks. Similarly, several studies showed that the diabetic rats had significantly lower weight gain than the controls [38, 39 &40]. In the study of Howarth et al., [39], they found that body weight in diabetic rats declined from 271 g before the administration of STZ to 238 g at 30 days after STZ treatment, whereas the body weight of control rats increased significantly from 247 to 314 g. Moreover, AL-Rawi [40] found at the end of the experiment that control rats gained about 38.91 % of the original body mass whereas the diabetic rats lost about 30.82%. A decrease in body weight of diabetic rats is possible due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control. Due to insulin deficiency protein content is decreased in muscular tissue by proteolysis [41]. The diabetes caused by streptozotocin administration increases fat mobilization in skeletal muscle [42], inducing significant weight loss [43] as was observed in the present study. Liver steatosis is a well-known pathology in severely obese patients and is especially associated with visceral adiposity and diabetes, It may progress in some patients to steatohepatitis and cryptogenic cirrhosis The histological mechanism of fatty liver disease (FLD) is yet not understood [44]. In many of obese people, increase of hepatic triglyceride levels, causes hepatic steatosis [45]. The aim of this study was to investigate the protective effects of N.S oil on STZ induced diabets histologically, in order to achieve this objective; diabetic rat model was constituted by means of STZ injection. It was claimed that lobular inflammation, pericellular fibrosis, portal fibrosis, hepatocellular ballooning, occurs in consequence of oxidant stress and mitochondrial dysfunction in FLD [46].

Current study indicated microvesicular fat globules and reduced glycogen content in study groups' slides and orientation of hepatocyte plates was corrupted, this event may have occurred as a result of oxidative damage in hepatocellular proteins, Abraham P. et al. have mentioned the same in their study [47] or necrotic changes in hepatocytes. The predictable fatty degeneration would first be detected. Moreover, this degeneration may point that liver damage could widen from around of lobules towards central area. But *MacDonald et al.,* [48] reported that degeneration first begins in hepatocytes of third zone. In this study, these decreases in glycogen contents of hepatocytes were determined mostly by Periodic Acid Schiff (PAS) method. Further more, there were no hepatocytes with dark PAS (+) stained cytoplasm.

Conclusion

The results of this study indicate that the oil of N.Sativa possesses hypoglycemic and antioxidant effects in STZ-induced diabetic rats and suggest that this oil may be a useful supplemental remedy in diabetes.

References

1- **Wild S, Roglic G, Green A, Sicree R and King H. (2004):** Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care, 27(5):1047- 1053.

2- **Kapoor S. (2009):** Emerging clinical and therapeutic applications of Nigella sativa in gastroenterology. World J. Gastroenterol., 15: 2170- 2171.

3- **Dorr R. T. and Fritz W. L. (1980):** Cancer chemotherapy. Hand book. London, Kinapton. Pp. 632-37.

4- **Punithavathi V R, Anuthama R and Prince P S (2008):** Combined treatment with naringin and vitamin C ameliorates streptozotocin-induced diabetes in male Wistar rats. J. Appl. Toxicol., 28(6):806-13.

5- **Fadillioglu E, Kurcer Z, Parlakpinar H, Iraz M, and Gursul C (2008):** Melatonin treatment against remote open injury induced by renal ischemia reperfusion injury in diabetes mellitus. Arch. Pharm. Res., 31(6):705-12.

6- **Gu D, Arnush M and Sarvetnic N (1997):** Endocrine/exocrine intermediate cells in Streptozotocin treated Ins-IFN-gamma transgenic mice. Pancreas, 15(3):246-50.

7- **Magee P. N and Swann P. F (1969):** Nitroso. compounds. Br. Med. Bull., 25:240-44.

8- **Piyachaturawat P, Poprasit J, Glinsukon T and Warichanon C (1988):** Gastric mucosal lesions in Streptozotocin diabetic rats. Cell Biol. Intern. Rep., 12(1):53-63.

9- **Piyachaturawat P, Poprasit J and Glinsukon T (1990):** Gastric mucosal secretions and lesions by different doses of Streptozotocin in rats. Toxicol. Let., 55:21-9.

10- **Arkkila P E, Koskinen P J, Kantola I M, Ronnemaa T, Seppanen E and Vikari J S (2001):** Diabetic complications are associated with liver enzyme activities in people with type-1 diabetes. Diabetes Res. Clin. Pract. ,52(2):113-8.

11- **Ramesh B, Viswanathan P and Pugalendi K. V (2007):** Protective effect of Umbelliferone on membranous fatty acid composition in streptozotocin-induced diabetic rats. Eur. J. Pharmacol., 566(1-3):231-9.

12- **Ali B. and Blunden G (2003):** Pharmacological and toxicological properties of Nigella sativa. Phytother Res., 17: 299-305

13- **Meral I, Yener, Z, Kahraman T and Mert N (2001):** Effect of Nigella sativa on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. J. Vet. Med. Physiol. Pathol. Clin. Med., 48(10): 593-599.

14-**Shalaby M and Khater O (2007):** Hepatoprotective and antioxidant effects of Nigella sativa oil on carbon tetrachloride induced liver damage in rats. J. Faculty Home Economic, Minufiya University, 16:32-41

15- **Zegarac J, Stipcevic T and Belscak A (2009):** Antioxidant properties and phenolic content of different floral origin honeys. J. Api Prod. Apimed. Sci., 1(2): 43-50

16- **Hajhashemi, V, Ghannadi A and Jafarabadi H. (2004):** Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phytother. Res., 18(3): 195-199.

17- **Banerjee S., Azmi A., Padhye S., Singh M., Baruah J., Philip P., Sarkar F. and Mohammad R. (2010):** Structure-activity studies on therapeutic potential of Thymoquinone analogs in pancreatic cancer. Pharm. Res., 27: 1146-1158.

18- **Kaleem M, Kirmani D, Asif M, Ahmed Q and Bano B. (2006):** Biochemical effects of Nigella sativa L seeds in diabetic rats. Indian J. Exp. Biol., 44(9): 745-748.

19- **Bhatti I, UrRehman F, Khan M.and Marwat S (2009):** Effect of Prophetic Medicine Kalonji (Nigella sativa L.) On Lipid Profile of Human Beings: An In vivo Approach. J, World Appl. Sci., 6(8): 1053-1057.

20- **Abdel-Fattah AM, Matsumoto K and Watanabe H. (2000):** Antinociceptive effects of Nigella sativa oil and its major component, thymoquinone, in mice Eur J Pharmacol. .,; 400(1):89-97.

21- **Bennett R A and Pegg A E (1981):** Alkylation of DNA in rat tissues following administration of Streptozotocin. Can. Res., 41:2786-90.

22- **Petlevski R, Hadzija M, Bajalo J. L and Juretic D (2006):** Effects of acarbose on alanine aminotransferase and aspartate aminotransferase activities in the liver of control and diabetic CBA mice. Acta. Pharma., 56:87-93.

23- **Bancroft JD and Stevens A (1996):** Theory and practice of histological techniques. 4^{,h}ed.Churchill Livingstone: Edinburgh.

24- **Seifter S, Dayton S, Novic B and Muntwyler E (1950):** The estimation of glycogen with the anthrone reagent. Arch Biochem., 25 (1): 191-200.

25- **Augusti K and Sheela C (1996):** Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia 52:115-120.

26- **Campos K, Diniz Y, Cataneo A, Faine L, Alves J and Novelli E (2003):** Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. Int J Food Sci Nutr 54:241–246.

27- **Kumar G and Reddy K. (1999):** Reduced nociceptive responses in mice with alloxan induced hyperglycemia after garlic (*Allium sativum*) treatment. Indian J Exp Biol., 37:662– 666.

28- **Jain R and Vyas C. (1975):** Garlic in alloxan-induced diabetic rabbits. AM J Clin Nutr., 28:684–685.

29- Ravi K, Ramachandra B and Subramanian S. (2004): Protective effect of *Eugenia jambolana* seed kernel on tissue antioxidants in streptozotocin induced diabetic rats. Biol Pharm Bull 27:1212-1217.

30- Rajasekaran S, Sivagnanam K and Subramanian S. (2005): Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. Pharmacol reports ,57:90–96.

31- Sekar D, Sivagnanam K and Subramanian S. (2005): Antidiabetic activity of Momordica charantia seeds on streptozotocin induced diabetic rats. Pharmazie, 60:383-397.

32- Al-Awadi F, Fatania H and Shamte U. (1991): The effect of a plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. Diabetes Res.,18(4):163- 168.

33- Al-Hader A, Aqel M and Hasan Z (1993): Hypoglycaemic effects of the volatile oil of *Nigella sativa* seeds. Int J Pharmacognosy ,31:96–100.

34- Szkudelski T. (2001): The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res., 50:537-546.

35- Burits M and Bucar F (2000): Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Res., 14:323-328.

36- Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A and Saija A. (2005): Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem .,89:549–554.

37- Jayaprakasha G, Negi P, Jena B and Jagan Mohan Rao L. (2007): Antioxidant and antimutagenic activities of *Cinnamomum zeylanicum* fruit extracts. J Food Comp Anal., 20:330–336.

38- Ananthi J, Prakasam A and Pugalendi K. (2003): Antihyperglycemic activity of Eclipta Alba leaf on alloxan-induced diabetic rats. Yale J Biol Med., 76:97-102.

39- Howarth F, Jacobson M, Naseer O and Adeghate E (2004): Short-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol 90:237-245.

40- AL-Rawi M (2007): Effect of *Trifolium* sp. flowers extracts on the status of liver histology of streptozotocininduced diabetic rats. Saudi J Bio Sci 14:21-28.

41- Vats V, Yadav S and Grover J (2004): Ethanolic extract of Ocimum sanctum leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. J Ethnopharmacol., 90:155–160.

42- Stearns S, Tepperman H and Tepperman J(1979): Studies on the utilization and mobilization of lipid in skeletal muscles from streptozotocin-diabetic and control rats. J Lipid Res 20:654–662.

43- Besse S, Assayag P, Delcayre C, Carré F, Cheav SL, Lecarpentier Y, and Swynghedauw B (1993): Normal and hypertrophied senescent rat heart: mechanical and molecular characteristics. Am J Physiol 265:H183- H199.

44- Adams LA, Sanderson S, Lindor KD and Angulo P (2005): The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. Hepatol., 42(1):132-8

45- Roden M, Anderwald C, Furnsinn C, Waldhausl W and Lohninger A (2000): Effects of short-term leptin exposure on triglyceride deposition in rat liver. Hepatology, 32 (5):1045-1059.

46- Brent A, Neuschwander-Tetri BA and Caldwell SH (2003): Nonalcoholic steatohepatitis: Summary of an AASLD Single Topic Conference Hepatology, 37(5):1202-19.

47- Abraham P, Wilfred G and Ramakrishna B (2002): Oxidative damage to the hepatocellular proteins after chronic ethanol intake in the rat. Clin Chim Acta., 325(1-2):117-25

48- MacDonald GA, Bridle KR, Ward PJ (2001): Lipid peroxidation in hepatic steatosis in humans is associated with hepatic fibrosis and occurs predominately in acinar zone 3. J Gastroenterol Hepatol., 16(6):599-606..