

Effect of *Nigella sativa* and *Carthamus tinctorius* L. Oils on Various Biochemical Parameters of Streptozotocin-induced Diabetic Rats

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Abstract

Effect of *Nigella sativa* and *Carthamus tinctorius* L. oils on some biochemical parameters was investigated in streptozotocin (STZ)-induced diabetic male Wistar rats. STZ-induced diabetic rats showed significant increases in the levels of blood glucose, triglycerides, cholesterol, low density lipoprotein LDL-cholesterol, creatinine, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while body weight, high density lipoprotein HDL-cholesterol, total protein levels were significantly decreased compared to normal rats. Administration of the tested oils to diabetic rats resulted in a significant decrease in blood glucose, triglycerides, cholesterol, LDL-cholesterol, urea, AST and ALT while body weight and HDL-cholesterol level was markedly increased after three weeks compared to untreated diabetic rats. The results of this study indicate that the tested oils possess hypoglycemic, hypolipidemic and antioxidant effects in STZ-induced diabetic rats.

Keywords: *Nigella sativa*; *Carthamus tinctorius* L.; Oil; Streptozotocin; Diabetes; Rats.

Introduction

Diabetes mellitus (DM) is one of the most common metabolic disorder worldwide, characterized by increase the blood glucose concentration due to deficiency of insulin secretion (Yoon *et al.*, 2006). This leads to oxidative stress, it appears mainly due to enhance the production of free radicals and decrease antioxidant substances (Hayoz *et al.*, 1998). The plants which contain antioxidants has been suggested as an integral treatment for blood glucose control (Bonfont-Rousselot *et al.*, 2000) as well as, the assessment of this natural origin of novel compounds is a right method for improvement of new antidiabetic treatment (Rchid *et al.*, 2004).

Nigella sativa (*N. sativa*) is a genus belonging to family Ranuncu-

laceae. A natural plant which is prevalently called with various names of dark cumin, dark seed and the seed of gift (Habatul-barakah). The seeds have been applied for several of years as a food additive and as a medical treatments (Halawani, 2009). *Nigella sativa* has been used as carminative, anthelmintics, diuretic as well as, treatment of cold, fever, antirheumatic and against poison of snakes and scorpions (Goreja, 2003; Arici *et al.*, 2005; Padhye *et al.*, 2008; Abdel-Sater, 2009). Nowadays, black seeds act as antibacterial, stimulate the immune system (Abel-Salam, 2012), decrease the blood pressure (Ali and Blunden, 2003), anti-inflammatory (El Mezayen *et al.*, 2006; Shuid *et al.*, 2012), anticancer (Mahmoud and Torchilin, 2013), antioxidant (Bourgou *et al.*, 2012; Umar *et al.*,

2012) and antidiabetic (Abdelmeguid *et al.*, 2010; Salama, 2012).

Carthamus tinctorius L. (safflower) (Compositae Family) is cultivated in Iran, the northwest India and some areas of Africa (Liu *et al.*, 2005). The therapeutic parts are blooms, seeds and the extracted oil from its developing seed embryos. The bloom extracts have been mentioned as antibacterial agent, enhance of peripheral blood circulation, prevention of blood platelet aggregation, decrease growth of skin tumor in mice and anti-inflammatory properties (Hiramatsu *et al.*, 2009). In addition, the antioxidant activity was reported (Choi *et al.*, 2010).

The study aimed to investigate if the administration of *N. sativa*, *Carthamus tinctorius* L. and *N. sativa* plus *Carthamus tinctorius* L. oils have useful effects on some biochemical parameters in STZ-induced diabetic rats.

Materials and methods

Oils: *Nigella sativa* and *Carthamus tinctorius* oils were purchased from the factory in Qus, Qena Governorate, Egypt. The factory used the dry method for extraction of the oil from seeds.

Chemicals: Streptozotocin (STZ) was purchased from Sigma Chemical Company (Germany). Freshly prepared STZ at a dose of 60 mg/kg dissolved in 0.01 M citrate buffer, pH 4.5. 25% Glucose and 0.9% NaCl.

Animals: 60 Wister-albino male rats, weighing 200-250 g with approximately 60 days old were used. The animals were put in stainless steel cages (30×50×25 cm³ dimension). Diabetes was induced by a sin-

gle intraperitoneal injection of streptozotocin at a dose of 60 mg/kg body weight. While control rats received only the saline solution (0.9% NaCl) in the same volume and through the same route. After 2 days, the fasting blood glucose levels were determined from tail blood samples by using an OneTouch Ultra® glucometer. Rats with blood glucose levels more than 277 mg/dL were considered diabetic and used for the experiment.

Experimental design and blood samples:

Rats were randomly divided into five groups as the following: Group1: Normal control (non-diabetic rats) received normal diet, Group2: STZ-Control (diabetic control rats) received the same diet given in Group 1, Group 3: diabetic rats received the same diet given in Group 1 plus 4 ml/kg body weight *N. sativa* oil, Group 4: diabetic rats received the same diet given in Group 1 plus 4 ml/kg body weight safflower oil, Group 5: diabetic rats received the same diet given in Group 1 plus a blend of 4 ml/kg body weight *N. sativa* oil and safflower oil (50/50). All groups received the treatment by intragastric catheter, one time per day for 21 days. Blood glucose level and body weight have been measured each week. At the end of the experiment, blood samples were collected from the orbital venous plexus of the rat and were centrifuged in a Nahita centrifuge model 2698 (GALILEO, Madrid, Spain) at 3000 rpm for 10 minutes to obtain a clear serum. The serum was stored at – 20°C for analyses of different biochemical parameters using commercial kits.

Biochemical assay: The measured parameters were total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C), by using DR-7000D Semi-Automatic Chemistry Analyzer (DIRUI, Changchun, China).

Result and Discussion

Effect of *N. sativa* and safflower oil on body weight

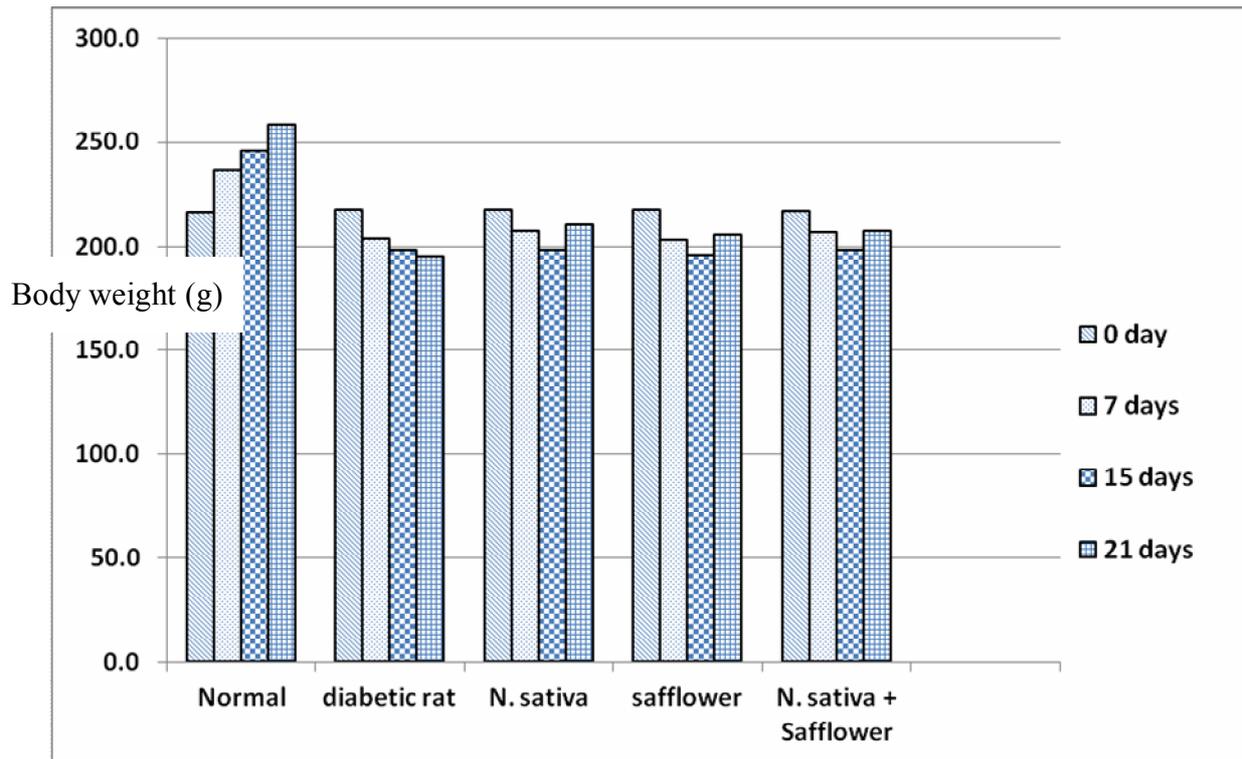
There were a progressive increase in the body weight of control group while, STZ induced diabetic rats have decreased in the body weight. As well as, the groups which were treated by *N. sativa* oil, safflower oil or *N. sativa* plus safflower oils had higher body weight changes (Table 1). Thus, several studies demonstrated that the diabetic rats had significantly lower weight gain than the controls (Ananthi *et al.*, 2003; Howarth *et al.*, 2005; AlRawi, 2007). A decline in body weight of diabetic rats is conceivable because of catabolism of fats and protein, despite the fact that the food consumption is more in diabetic rats than control. Besides, insulin deficiency lead to diminish the protein content in the muscle by proteolysis (Vats *et al.*, 2004). However, the increase the body gain in treated rats may be clarified by increased insulin production or increased food intake (Fernstrom and Fernstrom, 1993; Farouque and Meredith, 2003).

Effect of *N. sativa* and safflower oil on blood glucose level

The mean values of blood glucose of both control and experimental groups are showed in Table 2. STZ-incited diabetic rats presented a highly significant ($p < 0.001$) increase in blood glucose levels, about 497.3 mg/dl following three weeks contrasted with the controls. Intraoral injection of *N. sativa* oil, safflower oil and *N. sativa* plus safflower oils to diabetic rats resulted in a highly significant ($p < 0.001$) decrease in blood glucose levels of 133.9, 153.7 and 142.4 mg/dl respectively, after three weeks, compared to untreated diabetic rats. On the other hand, diabetic rats treated by *N. sativa* oil had the lowest blood glucose level. A significant increase in blood glucose level of diabetic rat is consistent with the finding of Augusti and Sheela (1996) and Campos *et al.* (2003) in rats, (Kumar and Reddy, 1999). Various studies showed that an assortment of plant extracts successfully down the blood glucose level (Ravi *et al.*, 2004; Rajasekaran *et al.*, 2005; Sathish Sekar *et al.*, 2005). Moreover, administration of *N. sativa* oil (Al-Awadi *et al.*, 1985; Al-Hader *et al.*, 1993) and safflower oil (Asgary *et al.*, 2012) had hypoglycemic effects in diabetic rats. However, the previous studies exhibited that the essential oils of safflower and *N. sativa* and their active constituents have demonstrated free radical scavenging and antioxidant effects (Burits and Bucar, 2000; Tomaino *et al.*, 2005; Asgary *et al.*, 2012).

Table 1. Effect of *N. sativa* and safflower oil supplementation on body weight (g)

Time	Normal	Diabetic rat	<i>N. sativa</i>	Safflower	<i>N. sativa</i> + Safflower
0 day	216.7	217.7	217.7	217.9	217.3
7 th day	236.7	203.7	207.6	203.6	206.9
15 th day	246.0	198.6	198.3	196.0	198.7
21 th day	258.6	195.4	210.4	205.6	207.6

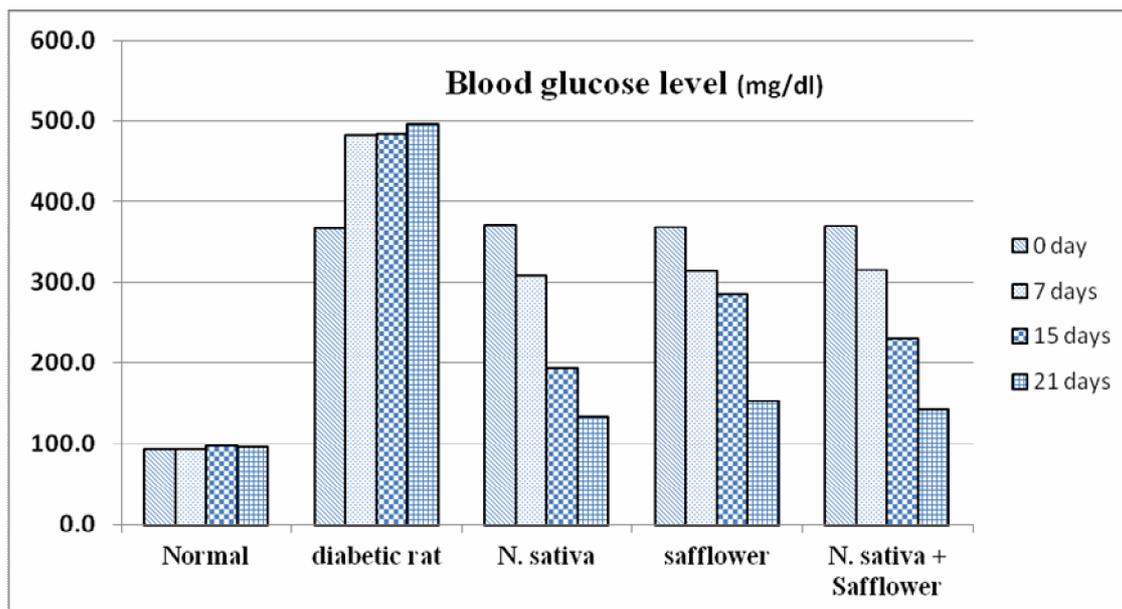


In view of previously mentioned reports, we propose that the conceivable mechanism of the studying oils action could be related to antioxidants help to recover from weak of glucose metabolism. As well as, The improvement of antioxidants in diabetics may prompt to protection of β cells of islet cells (Fararh *et al.*, 2002; Kanter *et al.*, 2003; Altan *et al.*, 2007; Abdelmeguid *et al.*, 2010; Alimohammadi *et al.*, 2013). This

protection was associated with a significant increase in insulin production and decrease in hepatic glucose production (Fararh *et al.*, 2004). Moreover, safflower oil is rich in a polyunsaturated essential fatty acid (80% Linoleic acid), there is evidence that free unsaturated fats assume an essential part in insulin production and glucose homeostasis (Rahimi *et al.*, 2014).

Table 2. Effect of *N. sativa* and safflower oil supplementation on blood glucose level (mg/dl)

Time	Normal	Diabetic rat	<i>N. sativa</i>	Safflower	<i>N. sativa</i> + Safflower
0 day	93.7	368.4	371.7	369.1	370.3
7 th day	94.0	484.3	309.0	315.0	316.1
15 th day	98.4	484.9	194.1	285.5	231.3
21 th day	96.6	497.3	133.9	153.7	142.4



Effect of *N. sativa* and safflower oil on lipid profile:

The changes in the lipid profile in control and experimental groups are illustrated in Table 3. There was a highly significant ($p < 0.001$) increase in the level of serum cholesterol, triglyceride and LDL in STZ-induced diabetic rats compared to the controls. In contrast, there was a highly significant ($p < 0.001$) decrease in the levels of serum HDL in STZ-induced diabetic rats. These results are similar to those obtained by Bolkent *et al.* (2004), Ravi *et al.* (2005), Singh *et al.* (2005) and Rajasekaran *et al.* (2006). Abnormal high concentration of serum lipids in diabetic rats are expected fundamentally to enhance in formation of free fatty acids from peripheral fat storage, since insulin blocks the hormone-sensitive lipase

(Pushparaj *et al.*, 2000). However, treatment of STZ-induced diabetic rats with *N. sativa* oil, safflower oil and *N. sativa* plus safflower oils resulted in a highly significant ($p < 0.001$) decrease in serum cholesterol, triglyceride and LDL levels, while a highly significant ($p < 0.001$) increased in serum HDL. These finding as forementioned by Zaoui *et al.* (2002) and Asgary *et al.* (2012). Accurate mechanism of *N. sativa* action is unknown, however, it has been demonstrated that volatile oil of *N. sativa* which has role in heart disease prevention (Abdel-Aal and Attia, 1993). Furthermore, it has strong antioxidant activity by removal of different free radicals (Badary *et al.*, 2003).

Table 3. Effect of *N. sativa* and safflower oil supplementation on lipid profile

Lipid profile	Normal	Diabetic rat	<i>N. sativa</i>	Safflower	<i>N. sativa</i> + Safflower
Cholesterol (mg/dl)	74.14	133.71	85.29	92.57	88.29
Triglyceride (mg/dl)	86.14	160.14	97.43	101.14	105.29
LDL (mg/dl)	33.34	85.11	43.94	53.06	47.37
HDL (mg/dl)	23.57	16.57	21.86	19.29	19.86

Effect of *N. sativa* and safflower oil on liver function tests

Serum AST and ALT activities, as markers of liver function were highly significant increased ($p < 0.001$) in the untreated diabetic animals in comparison with the control group. On the other hand, treatment of diabetic rats with *N. sativa* oil, safflower oil or *N. sativa* plus safflower oils leads to reduction of AST and ALT activities (Table 4). In-

crease the activities of AST and ALT may be induced due to liver dysfunction (Navarro *et al.*, 1993; Ohaeri, 2001), which gives an indication on the hepatotoxic effect of STZ. Moreover, administration of *N. sativa* oil, safflower oil or *N. sativa* plus safflower oils may inhibit the liver damage induced by streptozotocin (Al-Logmani and Zari, 2009). Consequently, decrease the activities of AST and ALT.

Table 4. Effect of *N. sativa* and safflower oil supplementation on serum AST and ALT (U/L)

Liver function tests	Normal	Diabetic rat	<i>N. sativa</i>	Safflower	<i>N. sativa</i> + Safflower
AST (U/L)	75.9	141.1	83.1	89.1	94.6
ALT (U/L)	48.1	76.3	54.1	59.7	56.9

Effect of *N. sativa* and safflower oil on kidney function tests

As cleared in Table 5, the diabetic rat showed significant increase in creatinine and urea compared to the control one. Intraoral treatment with *N. sativa*, safflower or *N. sativa* plus safflower oils to diabetic rats resulted in a significant ($p < 0.01$) decrease in blood urea level, in contrast there were no significant differences in creatinine level. Elevations

of blood urea and creatinine concentrations in diabetic rats may be due to reduction of serum protein and increase in circulating amino leads to the formation of large amount of ammonia which is inevitably changed to urea (Gawrońska-Szklarz *et al.*, 2003). Furthermore, the breakdown of amino acids during gluconeogenesis in the liver leads to increased urea production (Ganong, 2003).

Table 5. Effect of *N. sativa* and safflower oil supplementation on serum urea (mg/dl)

Kidney function tests	Normal	Diabetic rat	<i>N. sativa</i>	Safflower	<i>N. sativa</i> + Safflower
Creatinine (mg/dl)	0.47	0.56	0.51	0.54	0.53
Urea (mg/dl)	23.14	50.86	43.86	46.57	45.86

Effect of *N. sativa* and safflower oil on total protein

A highly significant ($p < 0.001$) decrease in blood total protein compared to the control. There were no

significant differences in the levels of protein after treatment of the diabetic rats by *N. sativa*, safflower or *N. sativa* plus safflower oils (Table 6). The pervious result is consistent with the

finding of (Peavy *et al.*, 1985; Wanke and Wong, 1991). This decrease might be because of suppression of oxidative phosphorylation which, result in decline of protein synthesis,

increase in the catabolic processes and reduction of protein absorption (Tragl and Reaven, 1972; Jefferson *et al.*, 1983).

Table 6. Effect of *N. sativa* and safflower oil supplementation on total protein (g/L)

	Normal	Diabetic rat	<i>N. sativa</i>	Safflower	<i>N. sativa</i> + Safflower
Total protein (g/L)	6.31	5.96	6.01	5.96	6.03

Conclusion

The results of this research appear that *N. sativa*, safflower or *N. sativa* plus safflower oils have antidiabetic and antilipidemic effects in diabetic rats so, recommend that these oils might be a valuable cure for diabetes.

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تأثير زيت حبة البركة وزيت القرطم علي بعض المعايير البيوكيميائية في الفئران المصابة بمرض السكري

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الملخص

تم دراسة تأثير زيت حبة البركة وزيت القرطم على بعض المعايير البيوكيميائية في ذكور الفئران المصابة بمرض السكري. أظهرت الفئران المصابة بداء السكري زيادة كبيرة في مستويات جلوكوز الدم ، الدهون الثلاثية ، الكولسترول ، كولسترول منخفض الكثافة ، الكرياتينين ، اليوريا ، بالإضافة إلى انزيمات الكبد. بينما انخفض بشكل ملحوظ وزن الجسم ، كولسترول عالي الكثافة و البروتين مقارنة مع الفئران العادية. تغذية الفئران المصابة بداء السكري بزيت حبة البركة وزيت القرطم ادي الي انخفاض كبير في نسبة الجلوكوز في الدم ، الدهون الثلاثية والكولسترول ، كولسترول منخفض الكثافة ، اليوريا وانزيمات الكبد في حين تم زيادة وزن الجسم ومستوى كولسترول عالي الكثافة بشكل ملحوظ بعد ثلاثة أسابيع مقارنة مع الفئران المصابة بالسكري غير المعالجة. تشير نتائج هذه الدراسة إلى أن الزيوت المختبرة لها القدرة علي خفض مستوى السكر والدهون في الدم في الفئران المصابة بداء السكري.