Clinicopathological Studies On The Effect Of *Nigella Sativa* Oil As Hypoglycemic Agent In Alloxan Induced Diabetic Rats

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ABSTRACT

The study was designed to evaluate the hypoglycemic potential of *Nigella sativa* oil (NSO) in alloxan induced-diabetic rats. A total of 65 male albino rats, 120-180 gm body weight were used. The rats were divided into 3 groups. Group 1 (15) rats were kept as a control. Group 2 (25) rats was injected intraperitonelly with alloxan monohydrate (150mg /kg b.wt). Group 3 (25) rats was injected intraperitoneally with alloxan monohydrate as in gp.(2) then administered with *Nigella sativa oil* (1ml/kg b.wt) orally. Serum samples were collected after 1, 2 and 6 weeks post diabetes induction.

Oral administration of *Nigella sativa oil* (1ml/kg b.wt) for 6 weeks after diabetes induction improved the glycemic status in alloxan induced diabetic rats. The serum level of insulin and high density lipoprotein (HDL) were increased while the serum glucose, cholesterol, triglycerides and low density lipoprotein (LDL) levels were significantly decreased in diabetic treated rats compared to the diabetic untreated rats.

INTRODUCTION

The black seed, *Nigella sativa*, is a plant that belongs to family *Ranunculaceae*. The seeds of *N. sativa* are the source of the active ingredients of the plant. It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavor in many countries. *N. sativa* volatile oil has recently been shown to possess 67 constituents, many of which are capable of inducing beneficial pharmacological effects in humans *(1)*. Diabetes Mellitus is a serious, complex metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion (β-cell dysfunction), insulin action (insulin resistance) or both *(2)*. Renewed attention to alternative medicine and natural therapies has stimulated new wave of research to look for more efficacious agents with lesser side effects *(3)*. Studies on the effect of *N. sativa* on blood glucose levels, in normal and diabetic animals, seem to be conflicting. Thymoquinone (TQ), the active principle of *Nigella sativa* plant oil has been shown to possess a hypoglycemic effect *(4,5)*. The oral administration of ethanol extract of N. sativa seeds (300 mg/kg b.wt/day) to streptozotocin (STZ) induced diabetes in rats for 30 days significantly reduced the elevated levels of blood lipids *(6)*.

MATERIAL AND METHODS

Experimental Design

A total of 65 apparently healthy male albino rats, 120-180 gm body weight were used in this study. The rats were divided into 3 groups. Group 1 of 15 animals were kept as a control. Group 2 of 25 rats was injected intraperitoneally with alloxan monohydrate (150mg /kg b.wt) **(7)**. Group 3 of 25 rats was injected intraperitoneally with alloxan monohydrate then administered orally *Nigella sativa* oil (1ml/kg b.wt) **(8)** for 6 weeks post diabetes induction.

Sample collection: Blood samples (5 samples from each group) were collected from retro-orbital venus plexus the eye after 1, 2 and 6weeks post diabetes induction in gps.1, 2 and 3. The blood was centrifuged for serum separation to be used for biochemical parameters estimation.

Biochemical studies: The serum level of glucose *(9)*, insulin *(10)* and total cholesterol *(11)* were estimated. Serum triacylglycerols was determined according to the method of *(12)*. Estimation of the HDL-cholesterol was carried out according to *(13)*. The serum LDL-cholesterol was calculated mathematically *(14)*.

Statistical analysis: The obtained data were statistically analyzed by F-test (one way ANOVA) in groups 1&2&3 *(15)*.

RESULTS AND DISCUSSION

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial /heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies *(16).* Alloxan is known for its selective pancreatic islet β–cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals *(17,18). Nigella sativa*, commonly known as black seed or black cumin, is used in folk medicine as a natural remedy for a number of diseases and conditions such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and gastrointestinal disturbances *(19).* In this study, increase in blood glucose level was observed on induction of diabetes mellitus on the rats. Our results clearly demonstrated that *(NSO)* at a dose of 1ml/kg B.W. for 6 weeks elicited a significant decrease in blood glucose level when compared with diabetic non treated rats. It may be attributed to the hypoglycemic effect of NSO and its active principle thymoquinone (TQ) through lowering the hepatic glucose production from gluconeogenic precursors (alanine, glycerol and lactate) *(20).* TQ up regulates the activities of hexokinase and glucose-6-phosphate dehydrogenase in hepatic tissues through the insulin release and enhance the utilization of the glucose for cellular biosynthesis which marked by significant decrease in the serum glucose level *(21).* Our results support many previous studies which reported that NSO is essential for regulation of blood glucose level in normal and diabetic rats. Several previous studies *(22-25)* showed that *Nigella sativa* potentially treated diabetic animals. Treatment of STZ induced diabetic rats with TQ for 4 weeks reduced the plasma glucose level *(26).* NSO succeeded in restoring insulin level towards the normal values which may be attributed to the regenerative effect of the oil on the β-cells of the pancreas and increase the sensitivity to secrete the insulin *(27).* Our result agreed with *(28),* diabetes was induced in rats by IP injection of streptozotocin (STZ), 8 wks later, the diabetic rats were weekly IP injected with *Nigella Sativa* (NS) (2ml/kg/day), for 4 wks, the NS treatment significantly increased the area of insulin immunoreactive β -cells in the diabetic rats *(28).* Moreover it has been reported that the simultaneous treatment with thymoquinone (2.5μM/kgm) increased the glucose stimulated insulin secretion *(29).* The treatment of STZ induced diabetic rats with TQ cause increase in the lowered serum insulin level *(24)* and significantly increase the insulin level through increase the immunoreactive insulin (IRI) *(26).*

Our result clearly demonstrated that the diabetic rats showed a highly significant increase in the level of cholesterol, triglycerides and LDL-c and highly significant decrease in the HDL-c. The abnormality high concentration of serum lipids in the diabetic is mainly due to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits hormones sensitive lipase. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on 3-hydroxy-3methylglutary coenzyme A (HMG COA) reductase, a key rate limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles *(30).* Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This result increased the production of cholesterol-rich LDL particles. *(31,32).* Alloxan-diabetic-rats showed a significant increase in the total lipid, total cholesterol, triglycerides, free fatty acids phospholipids, LDL and VLDL *(33).* Moreover cholesterol, triglycerides, HDL-c, LDL-c and VLDL-c were increased in streptozotocin induced diabetic rats *(34).* Also increased the total lipids and total cholesterol was recorded in alloxan induced diabetic rats *(35). Nigella sativa* oil and its active compound thymoquinone are able to lower the plasma cholesterol level in animals, probably because of its antioxidant activity *(36 ,37).* NSO treatment significantly decreased total cholesterol, TG and LDL-cholesterol and consequently increase the HDL-c in comparison with diabetic non treated group which showed significant increase in the previously mentioned parameters. While it restores HDL- cholesterol level to control value at 6 weeks post treatment. It may be contributed

to the NSO and its active constituent TQ which decrease the mobilization of fats from the peripheral adipose tissues and /or it improve the insulin secretion which activate lipoprotein lipase enzyme or it may has direct activating effect on the lipoprotein lipase enzymes which regulate the serum lipids or it may decrease the absorption of the diatory lipids. The reduction of the plasma level of cholesterol and LDL by TQ and *Nigella sativa* thymoquinone rich factor (TQRF) due to changes in the LDL-c which is probably due to the effectiveness of TQ and TQRF in regulating genes involving in cholesterol metabolism *(38)*.

The improvement of the level of HDL after daily treatment with NSO might be due to the increase in the activity of lecithin cholesterol acyle transferase which may contribute for the regulation of blood lipids *(39)*. Our result agree with the previous study *(40)* which showed that NSO lowered the blood cholesterol levels through its high content of β-sitosterol which inhibits the absorption of dietary cholesterol. Also TQ may protect against hyperlipidemia, associated with nephrotic syndrome, by significantly lowering the serum triglyceride and total cholesterol, triglyceride, total cholesterol and lipid peroxides in the kidneys in rats *(41)*. Dried petroleum ether extract of *Nigella sativa* seeds given to rats orally for 4 weeks significantly lowered the fasting plasma level of triglycerides and normalized HDL-c *(42)*. The oral administration of ethanol extract of *N. sativa* seeds (300 mg/kg body weight/day) to streptozotocin induced diabetes in rats for 30 days significantly reduced the elevated levels of blood lipids *(6)*. *Nigella sativa* (30mg/kg. body weight/day) added to the experimental diets for 20 weeks showed significant decrease in serum low density lipoprotein cholesterol level, and increase in serum high density lipoprotein cholesterol level *(43)*. *Nigella sativa* oil was effective as add on therapy in patients of insulin resistance syndrome *(44)*. The patients with insulin resistant syndrome treated with atorvastatin tablets (10 mg) once a day, metformin tablets (500 mg) twice day and *N. sativa* oil (2.5 ml) twice daily for 6 weeks, the NSO-treated patients showed a significant improvement by lowering the blood glucose, the total cholesterol (TC), TG and low density lipoprotein cholesterol (LDL-c) and increase HDL in *Nigella sativa* treated group when compared with the standard patients treated with atorvastatin or metformin. High levels of total cholesterol and more importantly LDL cholesterol in the blood are major coronary risk factors *(44)*.

It can be concluded from experimental findings that the levels of total serum cholesterol, total serum lipids and blood glucose levels which were actually raised in alloxan diabetic rats can be lowered by *Nigella sativa* oil. The hypoglycaemic and hypolipidaemic effects may be protective against the development of atherosclerosis, hyperlipidaemia and hyperglycemia common in diabetes mellitus.

Table 1. The glucose, insulin and lipid profile (mean values ± S E) in rats of all groups in different periods.

Parameters / Group	glucose (mg/dl)	insulin (µIU/ml)	cholesterol (mg/dl)	triglyceride (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
1^{1wk} Control	136.600 c ± 4.238	2.518 a ±0.033	107.598 c ±1.471	154.022c ±2.462	71.786 a ±0.922	4.908 c ±0.642
2^{1wk} alloxan	333.400 a ±2.088	0.874 b ±0.023	176.830 a ±2.868	209.550 a ±2.682	52.054 b ±2.098	83.162 a ±2.012
3^{1wk} alloxan+oil	309.600 b ±9.368	1.055 b ±0.105	135.600 b ±1.017	194.486 b ±1.804	57.886 b ±2.964	38.820 b ±2.672
1^{2wk} Control	116.598 c ±1.268	2.489 a ± 0.027	114.494 b ±1.063	105.964 c ±1.274	79.932 a ±1.740	15.096 c ±0.946
2^{2wk} alloxan	269.500 a ±4.009	0.933 c ± 0.006	140.930 a ±1.820	157.964 a ±2.242	51.596 c ±2.135	56.460 a ±0.640
3^{2wk} alloxan+oil	146.644 b ±5.540	1.168 b ± 0.012	96.564 c ±0.963	140.588 b ±3.271	66.598 b ±1.157	26.958 b ±1.412
1^{6wk} Control	101.020 c ±0.687	2.497 a ±0.025	100.800 a ±1.393	85.600 b ±١.913	80.700 a ±0.538	4.920 b ±١.067
2^{6wk} alloxan	146.740 a ±٠.401	1.074 c ±٠.005	95.600 b ±1.364	118.600 a ±٠.927	54.200 c ±1.562	19.560 a ±١.637
3^{6wk} alloxan+oil	129.300 b ±0.663	1.962 b ±0.008	94.000 b ±1.703	81.200 c ±٠.583	71.400 b ±٠1.077	6.240 b ±0.725

Means followed by different letters at the same column, at the same period were significantly different & the highest value was represented with the letter (a).

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الملخص العربي

دراسات باثولوجية إكلينيكية علي تأثير زيت حبة البركة كخافض للسكر فى الفئران المصابة بالسكر

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أجريت هذه الدراسة لتقييم تأثير زيت حبة البركة كخافض للسكر فى الفئران البيضاء المصابة بالبول أو الداء السكرى باستخدام مادة الالوكذان والفئران المستخدمة تجريبيا تم الحصول عليها من مزرعة حيوانات التجارب فى كلية الطب البيطرى جامعة الزقازيق .٦٥ فأر ابيض تم تقسيمهم الى ثلاث مجموعات كالتالي. المجموعة الأولي:وهي مكونة من ١٥ فأرا تم استعمالها كمجموعة ضابطة. المجموعة الثانية: وهي مكونة من ٢٥ فأرا تم حقنها بمادة الالوكذان داخل البروتينيم بجرعة(١٥٠ ملجم/كجم). المجموعة الثالثة: وهي مكونة من ٢٥ فأرا تم حقنها بمادة الالوكذان داخل البروتينيم ثم تجريعها يوميا بزيت حبة البركة(١ملى/كجم) عن طريق الفم لمدة ٦ أسابيع . أوضحت نتائج الباثولوجيا الإكلينيكية أن اعطاء زيت حبة البركة يحسن مستوى الانسولين و الدهون عالية الكثافة بالزيادة كما لوحظ انخفاض فى نسبة الجلوكوز والكوليستيرول والترايجلسريد و الدهون منخفضة الكثافة بالمقارنة بالمجموعة المصابة بالداء السكرى لذلك يوصى باستخدام زيت حبة البركة لمرضى السكر مع الادوية لتقليل الاثار الجانبية .