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Effects of Oral Administration of Water Extract of *Nigella sativa* on Serum Concentrations of Insulin and Testosterone in Alloxan-induced Diabetic Rats

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Abstract: The present study was designed to evaluate the role of a medicinal plant for management of diabetes instead of manufactured drugs that led to much complication. Water extract of *Nigella sativa* was investigated for hypoglycemic effect in diabetic rats in addition to some hormones related to diabetes mellitus. The extract induced significant reduction in serum glucose from (7.83±1.25) to (6.7±1.10), serum insulin increased from (0.55±0.08) to (0.65±0.06) and testosterone concentration significantly increased in treated diabetic animals (7.58±0.21) compared to diabetic animals (6.63±0.58). The results suggest the beneficial role of *N. sativa* as hypoglycemic agents and as a protective effect against pancreatic β-cells damage from alloxan induced diabetes in rats by decreasing oxidative stress and preserving pancreatic β-cells integrity and also suggest that the anti diabetic effect of *N. sativa* may be attributed to increased glucose metabolism. This in turn produces increase level of insulin in serum and testosterone concentration. Which might be related with the functional activity of the reproductive glands and the correlation with the functional state of the pancreatic β-cells.

Key words: *Nigella sativa*, pancreatic β-cells, testosterone, alloxan, hypoglycemic agents, insulin

INTRODUCTION

Diabetes Mellitus (DM) is one of the greatest medico-social problems of public health worldwide. It is one of the metabolic disorders, with a worldwide prevalence estimated to be in the range 1-5%. DM is a major cause of disability and hospitalization and it results in significant financial burden ($92 billion per year in US)[1]. By the year 2010, the total number of people worldwide with DM is projected to reach 239 millions. Regions with greatest potential are Asia and Africa, where DM rates could rise to 2-3-folds than the present rates[2].

DM leads to many complications, such as increasing the risk of developing arterial disease by two to six folds[3]. It has been suggested that the heart rate is higher, red and white blood cells counts lower in type diabetes than in non-diabetic[4,5].

In long-term diabetes, cardiomyopathy and congestive heart failure may also develop as a result of impaired left ventricular function[6]

DM significantly associated with hyperuricemia[7] and related to the disturbances in the secretion of sexual hormones, it has been suggested that there is an association between plasma levels of sex steroid hormones as testosterone and diabetes. Epidemiological observations suggest that low plasma level of testosterone is associated with increased risk for diabetes in men[8]. Furthermore, the plasma concentration of testosterone has been reported to be lower in men with diabetes than in nondiabetic subjects[9,10].

For the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents[11,12]. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown[13]. Many herbal medicines have been recommended for the treatment of diabetes *Trigonella foenum-graecum* L., *Ocimum sanctum* L., *Pterocarpus marsupial* L. and *Nigella sativa* have been shown to possess hypoglycemic activity in experimental animals[14-18].

N. sativa is a spice plant belonging to the family Ranunculaceae[19]. It is a medicinal plant that contains black seeds and has been used as a natural remedy for a variety of illnesses. *N. sativa* has more activities as bronchodilator[20], antibacterial[21], diuretic and hypotensive[22], liver necrosis[23], decreasing serum cholesterol, triglyceride and total lipids, increasing serum insulin, total liver glycogen[24] and raising the lower serum of T_3 concentration[25].

All the above prompted us to evaluate the effect of *N. sativa* extracts on blood glucose levels and serum concentration of insulin and testosterone in experimental rats.

MATERIALS AND METHODS

Preparation of extract: An extract of *N. sativa* seeds were prepared using the method described by

Table 1: Mean+SE of plasma glucose concentration of normal control, uncontrolled diabetic and *N. sativa* treated diabetic rats

Characteristic	Control	*N. sativa* treated*	Diabetic	*N. sativa* treated diabetic
Body weight (g)	186.0±9.88	198.0±10.88	178.0±13.0	177.6±6.3
Water drunk (mL day^{-1})	9.4±1.64	9.6±0.65	23.33±4.40	15.3±3.01
Urine excreted (mL day^{-1})	4.8±0.42	5.6±0.83	18.30±1.26	12.42±0.42
Protein (urine)	-	-	++	+
Ketone (urine)	-	-	+++	-
Glucose (urine)	-	-	+++	+
Blood glucose (nM L^{-1})	5.3±0.25	5.4±0.35	7.83±1.25	6.7±1.10

**N. sative* treated rates, - component is absent, + component is present in small amount, ++ component exists in large amount, +++ component exists in the largest level

Table 2: Plasma hormone concentrations of normal control, uncontrolled diabetic, *N. sativa* and *N. sativa* treated diabetic rats

Hormone	Control	N.S. treated	Diabetic	N.S. treated diabetic
Insulin (Mu L^{-1})	1.81±0.22	2.41±1.2	0.55±0.08	0.65±0.06
Testosterone (nM L^{-1})	14.04±0.54	18.69±0.40	6.63±0.58	7.58±0.21

Farida *et al.*[26]. *N. sativa* seeds were washed and air dried. An extract of *N. sativa* seeds in drinking water (5%) was prepared fresh daily by boiling the seeds (50 g) in drinking water (1000 mL) for 10 min and then filtering through 4 layers of surgical gauze to obtain the water extract used for the experiment.

Animals: White laboratory male rats (180-220 g) were housed in cages under standard laboratory conditions for at least 1 week before starting the experiments. Sixteen white male rats were divided equally into four experimental groups; control, diabetic, *N. sativa* treatment and *N. sativa* treated diabetic.

The animals of the control group were injected with physiological solution (Isotonic NaCl). The second group was made diabetic by interaperitoneal injections of 10% alloxan (Sigma chemical Co., St Louis, Mo. USA) (150 mg kg^{-1} body weight). After a fortnight, rats with marked hyperglycemia (FBS > 250 mg dL^{-1}) were selected and used for the study.

N. sativa treatment group was given the aqueous extracting of *N. sativa* seeds orally 20 mL kg^{-1} (substituted for drinking water) every day for 15 days.

The diabetic group was injected with 150 mg kg^{-1} of 10% alloxan to produce DM and then given the aqueous extract of *N. sativa* treated. The blood samples for all groups were taken from all rats to measure the serum concentration of glucose, insulin and testosterone.

Hormones determination: Serum insulin and testosterone were measured by radioimmunoassay methods (CEA-JRE-SORIN Firm, France).

RESULTS AND DISCUSSION

Effect of alloxan: Present results showed that 72 h after alloxan administration, serum glucose increased and

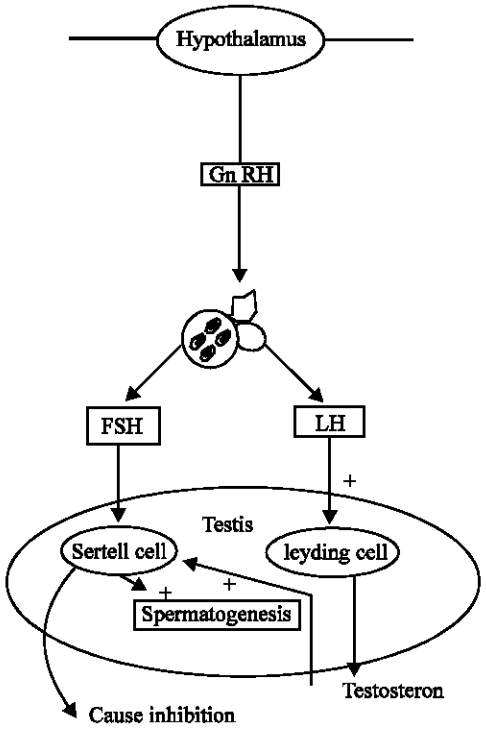

Fig. 1: Proposed mechanism of *N. sativa* action on serum testosterone by stimulating the gonad regulating cycle including the Hypothalamo-Pituitary Axis

serum insulin and testosterone decreased significantly. The increase of serum glucose (8.42±1.3, $p<0.05$) as shown in Table 1 and the decrease of insulin level (0.55±0.08 Mu L^{-1}) and testosterone level in blood (6.63±0.58 nM L^{-1}) as shown in Table 2 related to the effect of alloxan in alloxan treated group. During the experimental period we studied the symptomic complex of features in development of DM in rats after administration of alloxan, such as changes in appearance of an animal, the body weight and volume of water drunk, volume of urine excreted and determination of protein, ketones and serum glucose (Table 1) and the animals showed the following symptoms polydipsia, polyurinemia, weight loss, weakness and dehydration.

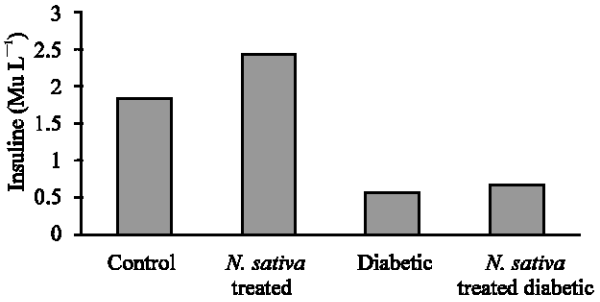

Fig. 2: Serum concentration of Insulin of normal control, uncontrolled diabetic and *N. sativa* treated diabetic rats, expressed as mean±SEM

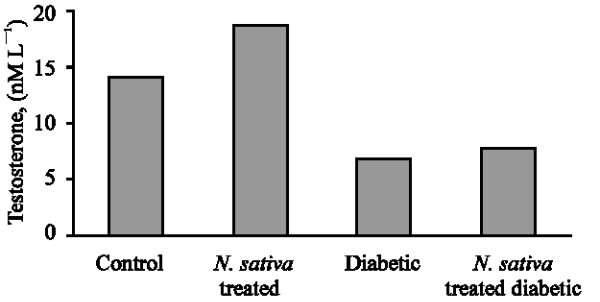

Fig. 3: Serum concentration of testosterone of normal control, uncontrolled diabetic and *N. sativa* treated diabetic rats, expressed as mean±SEM

Alloxan induces damage and death of pancreatic islet-cells in several experimental animal models, thus causing diabetes mellitus and decreasing the secretion of insulin. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species, Alloxan and the product of its reduction, dial uric acid; establish a redox cycle with the formation of super oxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells.

The relationship between testosterone and diabetes mellitus: In male rats, experimentally induced diabetes mellitus and reducing level of insulin ($p<0.05$) has been reported to be associated with reduced serum level of testosterone ($p<0.05$). While relative testosterone deficiency is associated with impaired carbohydrate metabolism. It was suggested that DM associated with decreasing sex accessory organ weight and gonadotropin levels and insulin restored testosterone levels to normal. Moreover, it was suggested that men with diabetes have lower testosterone levels and administration of physiological doses of testosterone improves glucose tolerance in these men and men with Coronary Artery Disease (CAD) have lower androgen levels than men with normal coronary arteries

Effect of *N. sativa* on blood glucose levels and on serum insulin levels: Treatment with *N. sativa* showed significant reduction in blood glucose ($p<0.05$) only on five days duration. It was reported by Abdel *et al.*[24] that *N. sativa* induced stimulation to insulin secretion. The hypoglycemic activity may be generally mediated through enhancement of peripheral metabolism of glucose and an increase in insulin release or may be due to an intestinal reduction of the absorption of glucose. The reduction induced by 200 mg dose seemed to be greater by the end of the experiment ($p>0.05$). However, their glucose concentrations were still higher (6.7±1.10 mM L^{-1}; Table 1) and insulin level was still lower (0.65±0.06 nM L^{-1}; Table 1, 2 and Fig. 2). The hypoglycemic effect of *N. sativa* reported here is in agreement with previous reports in normal and alloxan-induced diabetic rabbits and in human subjects. This might be attributed to the role of *N. sativa* that has been shown to provide a protective effect by decreasing lipid per oxidation and serum nitric oxide. Also by increasing antioxidant enzyme activity and exerts a therapeutic protective effect by decreasing oxidative stress and preserving pancreatic β-cell integrity. This is in good agreement with the findings that *N. sativa* treatment caused partial regeneration, proliferation of pancreatic β cells in alloxan-induced rats and the hypoglycemic action of *N. sativa*, could be partly due to amelioration in the β-cells of pancreatic islets causing an increase in insulin secretion.

Effect of *N. sativa* on serum testosterone levels: The results of analysis showed that the oral *N. sativa* treatment might increase the plasma testosterone level (18.69±0.40 nM L^{-1} $p>0.05$) compared to the control (14.04±0.54 nM L^{-1} $p>0.05$) and there were no significant different between third and fourth groups in testosterone level (Table 2 and Fig. 3). This might be explained as the hypoglycemic effect of *N. sativa* related with the increasing the level of testosterone dependent at the relation between insulin and testosterone. The increasing of testosterone level during experimental diabetes mellitus testifies that the changes in the function of reproductive glands can be noticed. These changes are expressed mainly a great decreasing of testosterone level after alloxan administration. At the same time the reproductive

glands are correlating with the functional state of the pancreatic islet-cells. It was reported that the low plasma level of testosterone has associated with increased insulin resistance for type 2 diabetes metes in male[8].

The results of recent study suggest that *N. sativa* treatment increased the level testosterone by stimulating the gonad regulating cycle including the hypothalamo-pituitary axis. This in turn regulates the amount of testosterone in the organism if the testosterone level in blood is low and the tests will signal the hypothalamus to release LHRH (Leutenizing Hormone Releasing Hormone). Thus the hypophysis releases gonadotropin, LH and FSH. Consequently, the interstitial cells of leydig that lie in small groups or individually between the seminiferous tubules of the tests activate the production of testosterone (Fig.1).

We suppose that *N. sativa* has a positive effect on preservation of testosterone homeostasis during alloxan-induced diabetes. It is possible that this influence occurs through the stimulation of interstitial cell receptors, which in turn secret testosterone.

It is concluded that oral *N. sativa* treatment might decrease the serum glucose and increased the levels of insulin from the pancreatic islet-cells and of testosterone from the interstitial cells of leydig cells in the tests

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