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Effects of Oral Administration of Water Extract of *Nigella sativa* on the Hypothalamus Pituitary Adrenal Axis in Experimental Diabetes

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Abstract: The present study was designed to evaluate the role of water extract of *Nigella sativa* on the hypothalamus-pituitary-adrenal axis in alloxan-induced diabetic rats. Forty male white rats were divided into four experimental groups control, diabetic. *N. sativa*-treated and *N. sativa*-treated diabetic. At the end of the experimental period (3 weeks), animals in all three groups were fasted for 12 h and blood samples were taken for the determination of glucose levels, serum concentrations of insulin, glucagon, corticosterone and Adrenocorticotropic hormone (ACTH) in four groups. It was found that water extract of *Nigella sativa* was investigated for hypoglycemic effect in diabetic rats and induced significant reduction in serum glucose from (19.83±1.25 Mmol L^{-1}) in diabetic group to (9.7±1.10 Mmol L^{-1}) in *N. sativa*- treated diabetic group. However the blood glucose still higher than the control and *N. sativa*- treated group, serum insulin increased from (0.54±0.22 Mu L^{-1}) in control group to (0.65±0.06 Mu L^{-1}) in *N. sativa* –treated group and still higher than control in *N. sativa* –treated diabetic (0.58±0,06 Mu L^{-1}), serum corticosterone increased in diabetic group (580 ± 22.36 nmol L^{-1})compared to control group (311±18.42 nmol L^{-1}) and decreased in *N. sativa*-treated (238±16.53 nmol L^{-1}) and in *N. sativa* treated diabetic group (378±19.65 nmol L^{-1}) and still higher than control. Serum Adrenocorticotropic hormone (ACTH) increased in diabetic group (20.72±2.42 pmol L^{-1}) compared to control group (13.82±1.83 pmol L^{-1}) and still lower in *N. sativa* -treated (10.64±13 pmol L^{-1}) and in *N. sativa* treated diabetic group (15.42±1.18 pmol L^{-1}) compared to diabetic group. The results suggest the beneficial role of *N. sativa* as hypoglycemic agents and as a protective effect against pancreatic ß-cells damage from alloxan induced diabetes in rats by decreasing oxidative stress and preserving pancreatic ß -cells integrity and also suggest that the antidiabetic effect of *N. sativa* may be attributed to increased glucose metabolism by increasing the serum concentration of insulin and inhibited the hypothalamus-pituitary-adrenal axis.

Key words: *Nigella sativa*, Alloxan, Insulin, corticosterone, adrenocorticotropic hormone (ACTH), glucagon

INTRODUCTION

Diabetes Mellitus DM represents a heterogeneous group of disorders that have hyperglycemia as a common feature[1,2]. Al though diabetes has long been considered a disease of minor significance, it is considered now as one of the main threats to human health in 21st century. Great changes in the human environment, behavior and lifestyle resulted in the raising rates of diabetes[3]. DM is consequence of defects in secretion, insulin action or both, which is translated into abnormalities of carbohydrate, fat and protein metabolism resulting in hyperglycemia[4,5]. Symptoms of chronic hyperglycemia include polypro, polydipsia and olyphagia as well as weight Loss. Although varying among patients, long term complications of Diabetes can also include changes in arteries (Atherosclerosis), basement membranes of small vessels (microangiopathy), Kidneys (nephropathy), retina (retinopathy) and nerves (neuropathy)[6-8]. DM leads to many complications, such as increasing the risk of developing arterial disease by two to six folds[9]. It has been suggested that the heart rate is higher, red and white blood cells counts lower in type diabetes than in non-diabetic[10,11]. There is some evidence that diabetics present a deficiency in mounting an inflammatory response, probably associated with severe reduction in insulin secretion rather than increased blood glucose levels[12,13]. This is still a controversial point as other investigators have suggested a direct correlation between hyperglycemia and the incidence of infection in diabetic patients[14].

DM significantly associated with hyperuricemia[15] and related to the disturbances in the hypothalamus-pituitary-adrenal axis, it has been suggested that there is an association between plasma levels of hormones (ACTH), corticosterone and diabetes[16]. Hyperactivation of the HPA axis of patients with diabetes mellitus has been reported previously, especially when poor glycemic control and ketoacidosis are present[17,18]. Both type 1 and type 2 diabetic patients have been characterized with

elevated circulating cortisol levels along with increased 24 h urinary free cortisol levels[19]. Moreover, diabetic patients have been shown to have disrupted circadian patterns of cortisol secretion, with elevated cortisol levels, during trough and normal or slightly elevated values during peak secretion[15]. Studies have revealed that increases in HPA activity in diabetic patients may be attributable to altered control of ACTH release from corticotrophs, as well as direct actions of CRH at the adrenal gland to release Cortisol independently of pituitary ACTH release[20]. In addition, both type 1 and type 2 diabetic patients exhibit greater incidences of nonsuppression of pituitary-adrenal activity, after glucocorticoid administration, compared with nondiabetic individuals[21]. This study suggests that hyperactivation of the HPA axis in diabetic patients may be attributable, in part, to decreased glucocorticoid-negative feedback sensitivity. However, the precise mechanism remains to be determined. Molecular regulation of the HPA axis has not been studied in humans. Increases in plasma glucocorticoid levels are beneficial, during times of stress, to aid in the mobilization of glucose stores from the liver and FFA from adipocytes, as well as to suppress further activity of the HPA axis. However, chronic exposure to elevated glucocorticoid levels is harmful[22-25]. Glucocorticoids inhibit glucose uptake in adipocytes and fibroblasts, decrease local cerebral glucose utilization and inhibit glucose uptake in hippocampal neurons *in vitro*. Prolonged exposure of hippocampal neurons to elevated glucocorticoid levels can lead to neurodegeneration or suppressed neurogenesis in the hippocampus, particularly in CA3 pyramidal neurons[26]. This may have important implications in neuropathologies associated with diabetes, especially in the areas of learning and memory and cognitive dysfunction[27].

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[28-32]. *N. sativa* is a spice plant belonging to the family Ranunculaceae[33]. 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[34] antibacterial[35] diuretic and hypotensive[36], liver necrosis[37], decreasing serum cholesterol, triglyceride and total lipids, increasing serum insulin, total liver glycogen[38] and raising the lower serum of T3 concentration[39]. The purpose of this study was to examine alterations in central regulation of the HPA axis during the early stages of alloxan-induced diabetes and to examine mechanisms involved in normalization of HPA activity with *N. sativa*- treated.

We hypothesize that central regulation of the HPA axis differs among normal, diabetic and *N. sativa*- treated diabetic rats. All the above prompted us to evaluate the effect of *N. sativa* extracts on blood glucose levels and serum concentration of insulin and effects on the hypothalamus-pituitary-adrenal axis by evaluating serum concentrations of corticosterone and Adrenocorticotropic hormone (ACTH), in experimental rats.

MATERIALS AND METHODS

Preparation of extract: An extract of *N. sativa* seeds were prepared using the method[40]. *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 (150-200 g) 6-8 weeks of age were housed in cages under standard laboratory conditions for at least 1 week before starting the experiments. Forty white male rats were divided equally into four experimental groups (control (n = 10), diabetic (n = 10), N. *sativa*- treated (n=10) and N. *sativa*-treated diabetic (n=10) rats. The animals of the control group were injected only with the same volume of isotonic NaCl as the diabetic groups received. The second group was made diabetic by intraperitoneal injections of 10% alloxan (Sigma chemical Co., St Louis, Mo, USA) dissolved in isotonic NaCl to induce diabetes (150 mg kg^{-1} body weight). Three days after alloxan injection DM was confirmed by the demonstration of hyperglycemia (Blood glucose 300 mg dL^{-1}), 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 21 days. The diabetic group was injected with 150 mg kg^{-1} of 10% alloxan daily 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 hormone and concentrations of adrenocorticotropic hormone (ACTH).

Serum Insulin, corticosterone, glocogon and adrenocorticotropic hormone (ACTH) were measured by radioimmunoassay (RIA), methods (CEA-JRE-SORIN Firm, France), using a commercial kit.

RESULTS AND DISCUSSION

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

decreased significantly. The increase of serum glucose ((19.83±1.25 Mmol L^{-1}) ($p<0.05$) (Table 1) and the decrease of insulin level (0.38±0.08 ng L^{-1}) (Table 2) and level corticosterone in blood (580±22.36 nmo L^{-1}) compared to control group (311±18.42 mol L^{-1}), decreased in *N. sativa* -treated (238±16.53 mol mL^{-1}) and in *N. sativa* treated diabetic group (378±19.65 mol mL^{-1}) and still higher than control. Serum adrenocorticotropic hormone (ACTH) increased in diabetic group(20.72±2.42 pmol L^{-1}) compared to control group (13.82±1.83 pmol L^{-1}) and still lower in *N. sativa* -treated (10.64±132 pmol L^{-1}) and in *N. sativa* treated diabetic group (15.42±1.18 pmol L^{-1}) compared to diabetic group (Table 2). During the experimental period 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. 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 dialuric acid; stablish a redox cycle with the formation of superoxide 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 hypothalamus-pituitary-adrenal axis *and diabetes mellitus*: Diabetes is associated with increased basal hypothalamo-pituitary-adrenal (HPA) activity and impaired stress responsiveness. Previously, we demonstrated that the HPA response to hypoglycemia is significantly impaired in diabetic rats. Plasma ACTH and corticosterone concentrations were significantly ($p<0.05$) higher in uncontrolled diabetic rats compared with normal and *N. sativa*-treated animals (Table 2). *N. sativa* decreased the glucagon concentration From (107.4±3.0 ng L^{-1}) ($p< 0.05$) in Uncontrolled diabetic rats to (75.3±8.5 ng L^{-1}) in *N. sativa*-treated animals. Others[41-44] have reported that diabetes mellitus represents a sustained stimulus to the HPA axis during the nadir of circadian activity. Although hyperactivation of pituitary-adrenal function has been demonstrated in both diabetic humans and animals, the underlying mechanisms responsible for these alterations are still unclear we have demonstrated that HPA dysregulation in early diabetes may be mediated, at least in part, by an increase in central drive at and/or above the level of the hypothalamic paraventricular nucleus[45]. We now provide evidence that HPA hyperdrive in diabetes is partially mediated by decreased glucocorticoid negative feedback sensitivity (as demonstrated by dexamethasone nonsuppression) and that impaired responsiveness of the diabetic HPA axis to stress, may be due to decreased pituitary and adrenal sensitivity. This latter point is evidenced by decreased responses to both CRH and ACTH challenge in diabetic animals. Despite significantly elevated basal plasma ACTH and corticosterone concentrations, the pituitary-adrenal response of diabetic rats to restraint stress was greatly diminished in comparison to control and treated diabetic animals. In a previous study, using the STZ-diabetic rat model, impaired responsiveness to a CRH challenge was correlated with a decreased number of CRH receptors in the anterior pituitary and it was suggested that this resulted from the hypersecretion of hypothalamic CRH[45].

Treatment with *N. sativa* showed significant reduction in blood glucose ($p<0.05$) only on five days duration. It was reported Unger[45] 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 and an decrease in glucagon release (75.3±8.5 ng L^{-1}) (Table 2) 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(9.7±1.10 Mmol L^{-1}) (Table 1) and insulin level was still lower (0.65±0.06 nmol L^{-1}) (Table 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 beta-cell integrity. This is in good agreement with the findings that *N. sativa* treatment caused partial regeneration, proliferation of pancreatic beta cells in alloxan-induced rats and the hypoglycemic action of *N. sativa*, could be partly due to amelioration in the beta-cells of pancreatic islets causing an increase in insulin secretion and causing a decreasing in glucagon secretion. Glucagon secretion is usually increased in cases of poorly controlled diabetes, mild or moderate diabetes results in normal or only moderately elevated glucagons levels[45]. Thus, the moderate diabetes

Table 1: Characteristics of rats after injection (Polydipsia, polyurinemia, weight loss, weakness and dehydration)

	Control	NS-treated*	Diabetic	NS-treated diabetic
Body weight (g)	186±9.88	198±10.88	178±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 in urine	–	–	+	+
Kitone in urine	–	–	+++	–
Glucose in urine	–	–	+++	+
Blood glucose (Mmol L^{-1})	5.3±0.25	5.4±0.35	19.83±1.25	9.7±1.10

*N. *sativa* treated rats. - component is absent, + component is present in small amounts, ++ component exists in large amount, +++ component exists in the largest level. Plasma glucose concentration of normal control, uncontrolled diabetic and NS – treated diabetic rats, expressed as mean±SEM

Table 2: Plasma hormone concentrations (Insulin Mu L^{-1}, Glucagon ng L^{-1} corticosterone nmo L^{-1}, ACTH pmol L^{-1}) of normal control, uncontrolled diabetic and N. *sativa*-treated diabetic rats

Hormone	Control	NS-treated	Alloxan-induced diabetic	NS-treated diabetic
Insulin (Mu L^{-1})	0.54±0.22	0.65±0.16	0.41±0.24	0.58±0.28
Glucagon (ng L^{-1})	101.0±3.7	82.3±9.4	107.4±3.0	75.3±8.5
Corticosterone (nmol L^{-1})	311±18.42	238±16.53	580±22.36	378±19.65
ACTH (pmol^{-1})	13.82±1.83	10.64±132	20.72±2.42	15.42±1.18

Results are expressed as Mean±SEM $p<0.05$

achieved may not have been sufficient to significantly elevate basal glucagon secretion. The decrease in basal glucagon secretion seen in the insulin-treated group was presumably caused by the direct suppressive actions of exogenous insulin on glucagon secretion[46].

The results of analysis showed that the oral *N. sativa* treatment might decrease the plasma corticosterone from (580±22.36 nmol L^{-1}) in diabetic group to (378±19.65 nmol L^{-1}) in *N. sativa* treated diabetic group and still higher than control, serum adrenocorticotropic hormone (ACTH) increases in diabetic group (20.72±2.42 pmol L^{-1}) compared to control group (13.82±1.83 pmol L^{-1}) and still lower in *N. sativa*-treated (10.64±132 pmol L^{-1}) and in *N. sativa* treated diabetic group (15.42±1.18 pmol L^{-1}) compared to diabetic group. This might be explained as the hypoglycemic effect of *N. sativa.* related with increasing the level of corticosterone and Adrenocorticotropic hormone (ACTH) dependent at the relation between insulin and these hormones. Normalization of pituitary-adrenal activity with *N. sativa* treatment seems to involve complex changes in the HPA axis, primarily through increased GR mRNA in the pars distalis, with normalization of the dramatically elevated hippocampal MR and hypothalamic CRH mRNA levels. In this study, significantly higher circulating plasma concentrations of both ACTH and corticosterone were observed in diabetic rats in the morning. Together, the endocrine data confirms hyperactivation of the HPA axis at the level of the pituitary and adrenal cortex under conditions of uncontrolled diabetes mellitus. More significantly, normalization of plasma insulin and glycemia, with *N. sativa* treatment, restored basal ACTH and corticosterone concentrations to control levels. These results indicate that glucocorticoid negative feedback sensitivity is decreased in the early stages of alloxan-diabetes and that sensitivity are restored with *N. sativa* treatment. In conclusion, results indicate that hyperactivation of the HPA axis in early allowance-diabetes is likely caused by both an increase in central drive and a decrease in glucocorticoid negative feedback sensitivity. Whereas impaired responsiveness to stress in induced-induced diabetic rats likely involves a decrease in sensitivity of the pituitary corticotrophin and adrenal cortex to CRH and ACTH, respectively. More importantly, normalization of pituitary-adrenal activity in induced-induced diabetic rats with *N. sativa* therapy can be attributed, in part, to restoration of insulin concentrations or pituitary-adrenal function. These impairments in HPA function in diabetes may contribute to cognitive dysfunction[26,47] decreased counterregulation to hypoglycemia[48] and an impaired ability to respond to stress.

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