THE EFFECT OF NIGELLA SATIVA L. SEEDS ON CERTAIN ASPECTS OF CARBOHYDRATES AND KEY HEPATIC ENZYMES IN SERUM OF RAT

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SUMMARY: In rats, an aqueous extract of the seeds of Nigella sativa was administered orally under light ether anesthesia for 7 and 14 days. Blood glucose, insulin level and key hepatic enzyme concentrations in serum an histopathological changes in the liver in both treatment groups for 7 and 14 days were compared with a control group which received normal saline under identical conditions for 14 days and with a group of normal animals. The data obtained indicate that blood glucose level exhibited significant decreases in both treatment groups after 7 and 14 days. Significant increases were recorded in insulin level in serum especially after 7 days post-treatment. Serum glutamate pyruvic acid transaminase (SGPT) concentrations were significantly higher following administration of N. sativa for 7 and 14 days when compared with the normal group but not when compared with the control group. On the other hand, serum glutamate oxaloacetic acid transaminase (SGOT) concentrations were not significantly different from one another. Serum gamma-glutamyl transpeptidase concentrations were significantly increased in both extract groups while serum alkaline phosphatase concentrations did not have a significant effect in both treatment groups after 7 and 14 days.

Histopathological examination of livers showed no evidence of degenerative changes following administration of N. sativa.

Key Words : Nigella sativa, blood glucose, hepatic enzymes.

INTRODUCTION

Seed of *Nigella sativa* L. (Family, Ranunculaceae) are poisonous to man in high doses. Its seed oil is a popular remedy for cough and bronchial asthma while the seeds are used for delayed menses, flatulence, respiratory oppression, as obtundent in toothaches and as diuretic, emmenagogue, carminative, vermifuge and antispasmodic (6,11,12,16). In addition to its possible galactogogue properties, *N. sativa* is considered to be potentially emetic and expectorant (9).

Since ancient times, plant remedies have been used to help to relieve diabetes. In the 6th century B.C., Sushruta, an Indian physician and surgeon classifying diabetes as a urinary disorder recommended plant remedies of 'salasaradi group'; an example of this group is *Gymnioma Sylvestre* (17). Subsequently many plants have been used throughout the world for the treatment of diabetes. In fact, more than 50 such plant extracts have been documented (7). In view of this wide usage, the WHO expert committee on diabetes mellitus (1980) has recently recommended that it is important to investigate the effect of agents of plant origin used in traditional medicine (1). It has later been reported that a plant mixture extract containing *N. sativa* is effective in lowering blood glucose and elevating insulin level in serum of rat.

We therefore planned to investigate the effects of extract of natural product of *N. sativa* seeds on blood glucose and insulin levels of serum in adult male albino rats.

During these investigations to evaluate the purported galactogogue activity of *N. sativa* a high inci-

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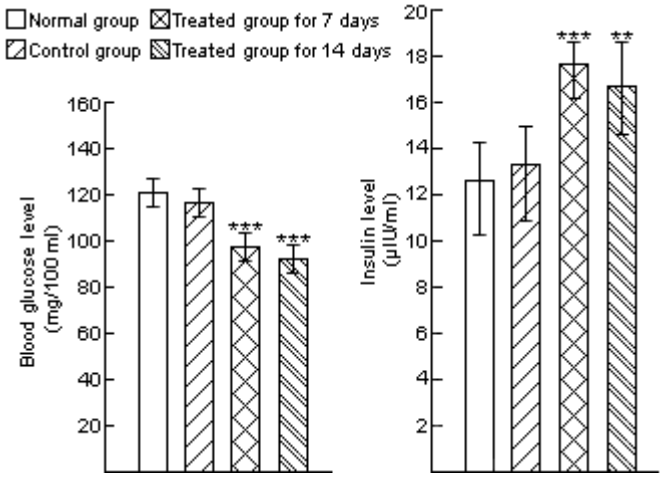
Figure 1: Serum glucose and insulin levels (values are the mean of each experimental group with the vertical bars representing standard errors) in adult male albino rats following 10 mg/kg oral administration of aqueous extract of the seeds of *Nigella sativa* for 7 days (n=9) and for 14 days (n=9) and normal saline for 14 days (control group, n=10) under light ether anesthesia and in a group of normal animals (n=10). (**) : p<0.01 vs. control and normal and (***) : p< 0.001 vs. control and normal.



dence of mortality was observed among the experimental animals, 1-2 weeks after completion of the experiment. In addition to its possible galactogogue properties, *N. sativa* is considered to have emetic and expectorant potential (9). Considering the extensive usage of this plant in medicine and my own observation of high mortality among experimental animals during my studies, it was thought necessary to investigate the possible hepatotoxicity of *N. sativa*.

MATERIALS AND METHODS

Preparation of the plant extract and treatment of animals

N. sativa seeds was purchased from the local market with a fair degree of quality assurance. These were washed to remove sand and other debris and air-dried. 300 g of these seeds were boiled in distilled water (1200 ml) for 90 min and filtered through muslin. The final volume of the filtrate obtained was 384 ml.

Adult, male albino rats (*Rattus norvegicus*) weighing 150-170 g each were used. They were randomly picked up from the experimental breeding station at Helwan. They were offered balanced standard maintenance diet with free access of water. Thirty-eight rats were selected for the study and were randomly divided into 2 groups of 9 each and 2 groups of 10 each.

Aqueous extracts of *N. sativa* (10 ml/kg) was administered by gastric intubation to rats in group 1 (N=9) for consecutive 7 days and group 2 (N=9) for consecutive 14 days, at a daily dose under light ether anesthesia. Group 3 (control group, N=10) received an equal amount of normal saline for 14 days under identical conditions and served as a common control group for the two treatment groups. Group 4 (normal group, N=10) was not administered by any thing.

At the end of the experimental period (i.e. 7 days for treatment group 1 and 14 days for treatment group 2, control group and normal group), animals were killed by cervical dislocation and 5 ml of blood was collected for estimation of glucose, insulin and key hepatic enzymes. Serum was separated and stored at -20°C until needed for analysis.

Methods

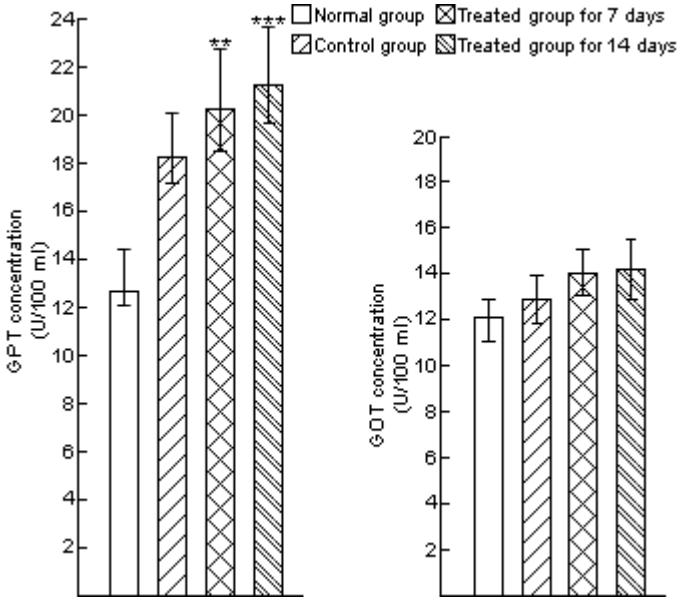
Glucose was determined using kits from Bio-Analytics Company. The level of insulin in blood serum was determined using the radio immunoassay (RIA) technique. RIA kits were provided by Diagnostic Products Corporation.

Gamma-glutamyl transpeptidase, alkaline phosphatase, glutamate pyruvic acid transaminase (GPT) and glutamate oxaloacetic acid transaminase (GOT) concentrations were measured using methods described by (10,14,15) respectively. Livers were harvested for histopathological examinations.

Statistical Analysis

Levels of glucose, insulin and enzymes concentrations in blood serum in the two treatment sub-groups and in the con-

Figure 2: SGPT and SGOT concentrations (values are the mean of each experimental group with the vertical bars representing standard errors) in adult male albino rats following 10 mg/kg oral administration of aqueous extract of the seeds of *Nigella sativa* for 7 days (n=9) and for 14 days (n=9) and normal saline for 14 days (control group, n=10) under light ether anesthesia and in a group of normal animals (n=10). (**) : p< 0.01 vs. normal; (***) : p< 0.001 vs. normal.



trol group were compared using the student's t-test. Further, these data in the treatment groups and in the control group were also compared with the data for normal rats, again using the student's t-test. Significance of data was calculated at P value <0.01 and at P value <0.001.

RESULTS

Blood glucose

The blood glucose levels (mean±S.E.M.) were 95.97±8.11, 90.15±7.02,115.13±5.11 and 120.02±6.05 mg/100 ml in groups treated with *N. sativa* for 7 and 14 days and in the control and normal groups, respectively. These serum levels in the treatment groups were significantly (p<0.001) decreased following administration of the oil relative to both the control group and the normal groups (Figure 1).

Insulin

The level of insulin in serum (mean ± S.E.M.) were 17.52±1.10, 16.51±1.95, 13.12±1.80 and 12.49±1.72 μIU./ml in the two treatment groups, control group and in normal group, respectively. Thus, the serum insulin levels following administration of *N. sativa* for one and two weeks were significantly higher (p<0.001) and (p<0.01) respectively when compared with the control and normal groups (Figure 1).

Further, there were no significant differences in regard to blood glucose and insulin levels between the control and the normal groups indicating that the transient but chronic ether administration for 2 weeks experienced by the control group did not affect glucose and insulin levels.

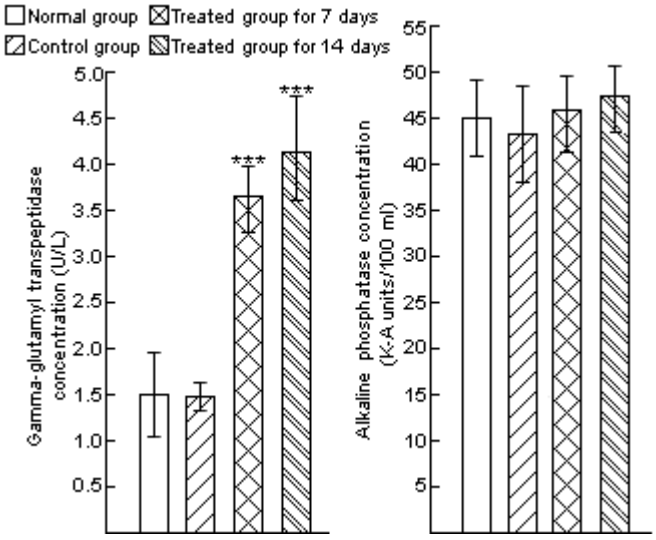
SGPT

SGPT concentrations (mean±S.E.M.) were 20.96 ±2.35, 21.98±2.05, 18.89±1.70 and 13.21±1.07 U/100 ml in the groups treated for one and two weeks and in the control and normal groups, respectively. SGPT concentrations were significantly higher following administration of *N. sativa* for 7 days (p<0.01) and for 14 days (p<0.001) when compared with the normal group but not when compared with the control group (Figure 2).

SGOT

SGOT concentrations (mean±S.E.M.) were 14.01± 1.11, 14.12±1.36, 12.78±1.27 and 11.99±0.89 U/100 ml

Figure 3: Serum gamma-glutamyl transpeptidase and alkaline phosphatase concentrations (values are the mean of each experimental group with the vertical bars representing standard errors) in adult male albino rats following 10 mg/kg oral administration of aqueous extract of the seeds of *Nigella sativa* for 7 days (n=9) and for 14 days (n=9) and normal saline for 14 days (control group, n=10) under light ether anesthesia and in a group of normal animals (n=10). (***) : p< 0.01 vs. control and normal.



in the groups treated with *N. sativa* for 7 and 14 days and in the control and normal groups, respectively and hence were not significantly different from one another (Figure 2).

Gamma-glutamyl transpeptidase

Serum gamma-glutamyl transpeptidase concentrations (mean±S.E.M.) were 3.60±0.42, 4.11±0.55, 1.47±0.19 and 1.51±0.42 U/1 in groups treated with *N. sativa* for one and two weeks and in the control and normal groups, respectively. These serum concentrations were significantly ($p<0.001$) increased following administration of oil for 7 and 14 days relative to both the control and normal groups (Figure 3).

Alkaline phosphatase

Serum alkaline phosphatase concentrations (mean ± S.E.M.) were 45.71±4.20, 47.12±3.99, 43.26±5.11 and 44.91±4.11 K-A units/100 ml in the two treatment groups and in the control and normal groups, respectively. Thus, the serum alkaline phosphatase concentrations following administration of *N. sativa* did not have a significant effect when compared with the control and normal animals (Figure 3).

Further, there were no significant differences in regard to gamma-glutamyl transpeptidase and alkaline phosphatase concentrations between the control and the normal groups.

DISCUSSION

The results of this study indicated that *N. sativa* given orally for 7 and 14 days to rats caused a blood glucose level to be decreased. Similar observations were also obtained by (1), who reported that plant mixture extract comprising of *N. sativa*, Myrrh, Gum olibanum, Gum asafetida and Aloe to have a blood glucose lowering effect. An investigation of plants extract comprising of *N. sativa* mechanism of action to establish whether the effect was exerted at the pancreatic and/or extra-pancreatic level (s) has shown that the anti-diabetic action was neither mediated through elevating plasma insulin levels nor by suppression of the intestinal absorption of glucose (1). In addition, (2) have shown that the anti-diabetic action of the plants

extract comprising of *N. sativa* is at least partly mediated through decreased liver gluconeogenesis. In the present work, the efficacy of *N. sativa* extract in reducing blood glucose may be attributed to either enhancing glucose oxidation and/or decreasing the rate of gluconeogenesis.

The present data indicate that *N. sativa* induced a marked increase of insulin level in serum during 7 and 14 days post-treatment. Similarly, (1) stated that plant mixture extract comprising of *N. sativa* induced increases in serum insulin in rats which was accompanied by a decrease in blood glucose level.

Although glucose is known as the major stimulator for insulin synthesis (13) and release (3) through influencing RNA synthesis ans post-transcriptional events in beta-cells (18) yet, (8) reported that when beta cells are stimulated the insulin is discharged, this is then followed by a refractory period in which responses to otherwise potent stimuli are dampened. Continued exposure to the stimuli then promotes a second sustained secretion.

The increase recorded in insulin level of serum in this work may result from stimulatory effect of *N. sativa* on the beta cells of islets of Langerhans (1).

The present study revealed the occurrence of marked elevation in SGPT enzyme activity after 7 and 14 days from *N. sativa* administration when compared with the normal group but not when compared with the control group. On the other hand, no significant effect of the plant seeds extract on SGOT enzyme activity. These observations are in accordance with the findings of (20), who demonstrated an increase in SGPT concentrations and no significant increase in the activity of SGOT in rats administered with *N. sativa* seeds or *Dregea volubilis* leaves.

GPT is a cytoplasmic enzyme found in very high concentrations in the liver. GOT is present in the cytoplasm as well as in the mitochondria and is less specific than GPT as an indicator of hepatic damage since it is rapidly inactivated (22). The oral administration of neither of plant extract used in this study nor ether anesthesia by itself appears to have a significant increase in GPT concentrations seen following oral administration of *N. sativa* when compared with the normal animals may indicate that administration of this plant extract when superimposed on ether anesthesia for 7 and 14 days may cause an increased release of this enzyme (20). However, neither oral administration of the plant extract nor ether anesthesia appears to have a significant effect on GOT concentrations.

The oral administration of *N. sativa* led to marked and significant increases in gamma-glutamyl transpeptidase enzyme activity of serum after 7 and 14 days post-treatment. On the other hand, no significant effect on alkaline phosphatase enzyme after administration of the plant extract. Similarly, (20) stated that *N. sativa* seeds and *Dregea volubilis* leaves induced increases in serum gamma-glutamyl transpeptidase concentrations while serum alkaline phosphatase concentrations were significantly increased following administration of only *Dregea volubilis*.

Serum gamma-glutamyl transpeptidase and alkaline phosphatase are membrane-bound enzymes and are released unequally depending on the pathological phenomenon. Elevation of serum gamma-glutamyl transpeptidase concentration is generally regarded as one of the most sensitive indices of hepatic damage (19).

In view of the above considerations, the increase in serum gamma-glutamyl transpeptidase enzyme concentration in this study might reflect hepatic damage under the action of the repeated high doses from *N. sativa* oil.

Serum alkaline phosphatase concentrations are known to be markedly elevated in cholestasis and to be minimally increased in chronic hepatocellular disease (22). No significant difference after oral administration of the *N. sativa* extract was recorded in this study. The significantly increased serum alkaline phosphatase concentrations seen following administration of *Dregea volubilis* indicate a difference in toxicity between this plant and *N. sativa* (20).

Histopathological examination of livers showed no evidence of degenerative changes in hepatocytes in animals treated with *N. sativa* for one and two weeks, in control animals or in normal animals.

Central veins and the sinusoids around them were dilated in animals treated with *N. sativa* for 7 and 14 days and in some control animals while the portal veins and the sinusoids around them were dilated in most

treated animals for 7 and 14 days and in most control animals. Dilatation of central veins, portal veins or sinusoids was not seen in any of the normal animals. It may be that ether anesthesia for 14 days led to dilatation of the central veins and surrounding sinusoids while oral administration of *N. sativa* enhanced this effect. The prevalence of dilatation of portal veins and surrounding sinusoids was comparable in the two treatment groups and in the control group indicating that this may have been caused by ether anesthesia for 14 days and that the oral administration of the plant extract did not have a superimposing effect.

Infiltration by mononuclear chronic inflammatory cells was observed in the vicinity of the portal tracts in rats treated with *N. sativa* for 7 and 14 days, in control animals and in 2 out of 10 normal animals. These observations are in accordance with the results of (20), who also stated that this finding is rather interesting as infiltration by inflammatory cells was not seen in rats treated with *Dregea volubilis*; yet, they showed hepatic degeneration. Is it that the animals treated with *N. sativa* showed an inflammatory response which prevented toxic material from causing degenerative changes in the hepatocytes? However, it is difficult to explain a possible defensive role of these inflammatory cells as these were also seen in the control animals.

Kupffer cells laden with bile pigments were seen in some animals treated with *N. sativa* after 14 days and in some control animals.

(4,5) have previously reported that some plant toxins may damage the hepatic cells at the molecular level without causing overt histopathological changes.

In my studies, a significant elevation of certain key hepatic enzymes and varying degrees of histopathological changes in the liver were documented following oral administration of boiling water extracts of seed of *N. sativa*. An increase in gamma-glutamyl transpeptidase and GPT concentrations in the absence of hepatocyte degeneration were observed following oral administration of *N. sativa* extract and this suggests that these enzymes may have been released due to hepatocellular damage caused at the molecular level. However, an enzyme-inducing effect of *N. sativa* extract cannot be ruled out.

In view of these preliminary findings, the repeated use of this medicinal plant as therapeutic agent should not be encouraged.

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