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PRELIMINARY STUDY ON THE EFFECT OF NIGELLA SATIVA L. EEDS ON HYPOGLYCEMIA

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SUMMARY

The hypoglycaemic effect of Nigella sativa L seeds, which is traditionally used in Egypt for the treatment was studied using alloxan-diabetic adult male albino rats. It was supplemented at 1%, 2% and 10% of the diets. The gain in body weight ; relative liver and kidney weight ratio; serum glucose; serum total lipids; total cholesterol; triglycerides; serum total protein, albumin, globulin; serum GOT and GPT activity; serum uric acid and urea were determined. The obtained results revealed that, dietary. Nigella feeding significantly increased serum glucose by about 250% over the control level. There was significant increase in all serum previous parameter.

It was concluded that, *Nigella sativa L.* seeds had no hypoglycemic effect.

INTRODUCTION

Nigella sativa L (Ranunculaceae) is a herbaceous plant that grows in Mediterranean countries and is also cultivated in India and Pakistan, and the seed is used for sprinkling on bread, flavoring vinegar and as a carminative and stomachic in native medicine (Redgrove, 1933; Chopra, et al., 1956; Nadkarni, 1976). The seeds are used by the Egyptians as a diuretic, carminative and flavoring agent, by the Syrians for cheese flavoring and by the Armenians for flavoring cheese and bakery products. The expresed oil has been used for treatment of asthma (Muschler, 1912).

No work has previously been carried out on its hypoglycemic effect. So, the study presented here (first study) had concentrated to evaluate *Nigella sativa L.* Seeds which are traditionally used in Egypt, to establish its activity as a hypoglycaemic agent and to evaluate the effect of its administration on lipids metabolism; liver and renal function of normal and alloxanized diabetic rats.

MATERIAL AND METHODS

I. Material:

Nigella sativa L. Seeds were obtained from fiel crops Research Institute, Agricultural Researc Centre, Ministry of Agriculture. Fatty aci composition of Nigella sativa L. seed oil presented in Table 1.

II. Animals:

Forty adult male albino rats, Sprague-Dawley strain, weighing between 96-145g were used. They were obtained from Helwan breeding farm, Cairo Egypt. The animals were divided into five groups and housed individually in stainless steel cages with wire mesh bottoms in a room maintained at 25-30C with about 50% relative humidity. The room was lighted on a daily photoperiod of 12 h light and dark.

III. Experimental diets:

Adult male albino rats were divided into five groups (eight animals each) and four groups were injected intraperitoneally with alloxan (150 mg/kg body weight) as described by Lazarow and Palay (1954), while the fifth group was kept as negative control All animals were fed one of five diets as follows:

1. Rats fed on basic diet (negative control)
2. Injected rats fed on basic diet (positive control).
3. Injected rats fed on basic diet + 1% *Nigella sativa L.* Seeds.
4. Injected rats fed on basic diet + 2% *Nigella sativa L.* Seeds.
5. Injected rats fed on basic diet + 10% *Nigella sativa L.* Seeds. Food and Water were provided ad libitum. Body weight gain was recorded. Composition of the basal diets is shown in table 2. The design, treatment and feeding duration of each experiment are indicated within the tables of results.

Analytical Procedures:

Blood samples were obtained from each rat from the orbital venous plexuses by capillary tube every 48 hours intervals, for 9 days to determine the blood glucose level. At the end of 9 d. rats were anesthetized with ethyl ether. Incisions were made into the abdomen and blood samples were obtained from the portal vein and left to clot and centrifuged at 1300 X g for 15 min at 4C to obtain serum. Liver and Kidney were excised, rinsed in chilled saline solution, then blotted on filter paper, weighed separately to calculate the absolute and relative organ weight.

Serum glucose level was analyzed by colorimetric procedures kits developed by Diamond Diagnostics Kits. Cairo Egypt according to (Trinder, 1969).

Serum total lipids were analyzed by enzymatic colorimetric procedures kits developed by the Egyptian American company EAC. for laboratory services. Cairo, Egypt according to (Thannhouser, 1958), for cholesterol, kits (Cat No. 290) supplied by Randox laboratory limited, Ireland according to (Allian, et al., 1974). Triglycerides were determined by using colorimetric procedures kits supplied by (Randox laboratory limited Ireland) according to (Wahlefeld, 1974). Serum total protein analyzed by using colorimetric method kits no. 3327, E. Merck, Frankfurter str. 250 D-6100. Darmstadt I), according to (Sunderman et al., 1958).

Albumin/globulin ratio (Alb/Glob) was calculated. Serum levels of glutamic oxaloacetic transaminase (GOT) and (GPT) were analyzed by enzymatic colorimetric procedures kits supplied by (Biocon Diagnostik GmbH, Burbach Germany), according to the (Reitman and Frankel

Table 1:Fatty acid composition of *Nigella sation L.* seed oil*.

Table (1) : Fatty acid composition of *Nigella sativa L. seed oil.**

Fatty acids		*Percent composition*
Myristic	(14 : 0)	0.16
Palmitic	(16 : 0)	12.08
Stearic	(18 : 0)	3.11
Oleic	(18 : 1)	24.64
Linoleic	(18 : 2)	56.12
Linolenic	(18 : 3)	0.70
Eicosadienoic		2.53
Total saturated Fatty acids		15.35

* *Babayan, et al. (1978)*

1957). Serum level of uric acid was analyzed by colorimetric procedures kits supplied by (Pasteur lab., 40 Manial street. Cairo, Egypt), according to (Henry, 1974). Whilst urea was analyzed by Kits no. 3334, E. Merck, Frankfurter str, 250, D-6100 Darmstadtl, according to (Fawcett and Scott, 1960).

Statistical analysis was done by completely randomized design in factorial arrangement (F-test), least significant difference (L. S. D.) was used for comparing treatment means (Snedecor and Cochran, 1980).

1. The mineral mixture in g/200 g diet was as follows $CaCO_3$, 60.0; $K2HPO_4$, 64.5; $CaHPO_4$, H_2O , 11.5; KI, 0.16; $MnSO_4$ $4H_2O$, 1.0; Z nCl2 0.05; $CuSO_4$ $5H_2O$, 0.06; $MgSO_4$ $7H_2O$ 20.4;

Table (2): Composition of basal diets for rat experiments.

Ingredient	*Basal diet g/100 g diet*	*Basal diet + 1% Nigella sativa*	*Basal diet + 2% Nigella sativa*	*Basal diet + 10% Nigella sativa*
Corn starch	67.70	66.70	65.70	57.70
Casein	12.50	12.50	12.50	12.50
Corn oil	10.00	10.00	10.00	10.00
Fiber	5.00	5.00	5.00	5.00
Mineral mix[1]	3.50	3.50	3.50	3.50
Vitamin mix[2]	1.00	1.00	1.00	1.00
DL-methionine	0.30	0.30	0.30	0.30
Nigella sativa L. Seeds	--	1.00	2.00	10.00

NaCl, 33.5; Fe $(C_6H_5O_7)_2$ $6H_2O$, 5.5; Selenium, 0.002; Chromium, 0.4; sucrose, finely powdered to make 200 g, according to Hegsted, et al., (1941).

2. The vitamin mixture in mg/g of diet (except as noted) was as follows: Retinyl palmitate, 1000 I. U.; Cholecalciferol, 100 I. U.; DL a-tocopheryl acetate, 10 I. U., Menaquinone, 0.5; thiamin-HCI, 0.5; riboflavin, 1; Pyridoxine-HCI, 0.4; D-Calcium pantothenate, 4; niacin, 4; choline chloride 200; inositol, 25; para amino-benzoic acid, 10; cyanocobalamin, 0.002; biotin, 0.02; folic acid 0.2; sucrose, finely powdered to make gram, according to Campbell, (1961).

Table (3): Effect of feeding diets containing different levels of *Nigela sativa L.* seeds on growth rate of rats during 9 days.

Experimental Diet	*Initial wt. (g)*	*Final wt. (g)*	*Gain in wt. (g)*	*% change in wt.*
G_1. negative control	136.33	166	29.67	--
G_2. positive control	145.00	158.25	13.25	- 55.34
G_3. injected rats fed on 1% Nigella Sativa L. Seeds	96.44	111.55	15.11	- 49.06
G_4. injected rats fed on 2% Nigella Sativa L. Seeds	118.55	131.22	12.67	- 57.32
G_5. injected rats fed on 10% Nigella Sativa L. Seeds	125.63	137.5	11.87	- 59.99

RESULTS AND DISCUSSION

Clinical signs during the experimental period:

1- There were malaise, illness, anorexia, sluggish behaviour between all experimental rats.

2- The liver varied in colour from light red (G_3, G_4 and G_5) to dark red (G_2).

Weight gain:

There was a little effect on weight gain during the study (Table 3). Weight gains were almost similar for injected rats fed on the basic diet (positive control) and corresponding rats fed on basic diet containing 2% *Nigella Sativa L.* seeds, whereas *Nigella sativa L.* seeds (10%) fed rats gained the least weight (-59.99% from negative control). It is possible that the effects was solely due to differences in total *Nigella sativa L.* seeds content among the diets, and the animals found the experimental diet (10% unpalatable).

Relative Organs Weights:

There was a significant difference in relative liver weight among groups (Table 4). Rats fed *Nigella sativa L.* seeds at either 2% or 10% had lower relative liver weights than rats fed all other test diets, 2.92 and 2.56 respectively ($P<0.05$) (Table 4) whereas there was a slight insignificant ($P<0.05$) increase in kidney relative weights in rats fed diet (G_5) tan in those fed diet (G_2) (Table 4).

Administration of *Nigella sativa L.* seeds at 2% level was accompained by a marked decrease in the liver relative weight and the maximum decrease was noticed in the animals fed on 10% level. This marked decrease may be attributed to the fed 10% level. This marked decrease may be attributed to the reason which was mentioned previously concerning the animals found the experimental diet (10%) unpalatable, which may impair metabolic systems.

Table (4): Effect of feeding diets containing different levels of *Nigella sativa L.* seeds on organ/body weight ratio.

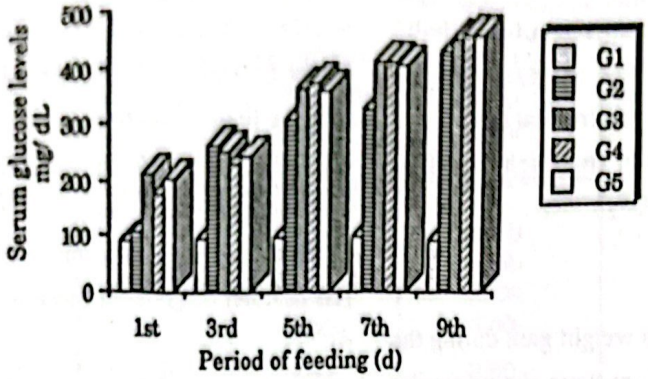
*Experimental Diets**	*Liver weight/ 100 g body weight*	*Kidney weight / 100 g body wt*
G_1. negative control	4.94 ± 0.50^{a}	1.25 ± 0.16^{a}
G_2. positive control	3.54 ± 0.31^{ba}	0.81 ± 0.12^{ba}
G_3. injected rats fed on 1% Nigella Sativa L. Seeds	3.16 ± 0.32^{ca}	0.79 ± 0.12^{ca}
G_4. injected rats fed on 2% Nigella Sativa L. Seeds	2.92 ± 0.41^{dab}	0.78 ± 0.14^{da}
G_5. injected rats fed on 10% Nigella Sativa L. Seeds	2.56 ± 0.40^{eabc}	0.84 ± 0.09^{ea}

* values are means ± SD, n = 8/ group.

abcde Means in the same column with different superscripts differ significantly ($p < 0.05$).

Fig. 1

Effect of feeding diets containing different levels of Nigella sativa L. seeds on serum glucose levels.



* **Normal value of serum glucose in rats :**
47.73-106.98 mg/100ml *(Burns and Lannoy, 1966)*

Serum Glucose:

Serum glucose was not altered by dietary treatments . Glucose levels sharply increased during the 3rd day to 9th day (Fig. 1).

Injection of alloxan caused a substantial increase of blood glucose level. Alloxan may have increased hepatic glycogenolysis or gluconeogenesis or decreased the rate of removal of glucose from bood by the tissues. These influences are due to the absence of an adequate amount of insulin. The alloxan acts directly promptly and specifically on the β-cells of the pancreatic islets. Within a week most of the beta cells have disappeared leaving little evidence of fibrosis or scarring in the islets tissue (Lazarow and Palay, 1954).

Serum Lipids:

Table 5 depicts the changes in serum total lipids; total cholesterol and triglyceride that occurred during the 9 days experimental period. Serum levels of total lipids and triglyceride remained almost as normal value in rats fed 2% and 10% *Nigella sativa L* seeds. Whilst 1% *Nigella sativa L.* Seeds feeding was accompained by higher serum total lipids concentrations, whereas serum triglyceride significantly increased slightly among treated groups when compared with normal value ($P<0.05$) Table 5.

The mechanism of the increasing of total lipids absorption by dietary 1%. *Nigella sativa L.* seeds is not fully understood. The presence of different quantities of fatty acids of varying chain lengths and degrees of saturation in the oil might play a role in total lipids absorption. Table 1. Most fatty acids in the body are esterified in triglycerides or phospholipids, which act as storage or transport vehicles for fatty acids. In the bood there is also a relatively small amount of unesterified or "free" fatty acids (FFA). The major source of circulating FFA is adipose tissue, which releases them after hydrolysis of its storage triglyceride; some FFA

Table (5): Effect of feeding diets containing different levels of *Nigella sativa L.* Seeds on serum lipids.

Experimental Diets	Serum Lipids		
	Total lipids* g/L	Cholesterol* mg/dL	Triglyceride* g/L
G_1, negative control	3.83 ± 0.62^{a}	102.26 ± 7.06^{a}	9.77 ± 0.76^{a}
G_2, positive control	7.23 ± 0.72^{ba}	176.18 ± 46.64^{ba}	12.36 ± 1.32^{ba}
G_3, injected rats fed on 1% Nigella Sativa L. Seeds	5.35 ± 0.90^{cab}	148 ± 59.18^{ca}	7.97 ± 1.69^{cab}
G_4, injected rats fed on 2% Nigella Sativa L. Seeds	3.11 ± 1.15^{dbc}	114.47 ± 38.44^{db}	7.67 ± 1.02^{dab}
G_5, injected rats fed on 10% Nigella Sativa L. Seeds	3.19 ± 0.53^{ebc}	116.01 ± 27.05^{db}	7.09 ± 1.92^{eab}

* Normal value of serum total lipids in rats : 310 ± 50 mg/100ml *(Miura, et al., 1989).*

* Normal value of serum total cholesterol in rats : 90 ± 14 mg/100ml *(Miura, et al., 1989).*

* Normal value of serum triglyceride in rats : 77 ± 14 mg/100ml *(Miura, et al., 1989).*

abcde Means in the same column with different superscripts differ significantly ($p < 0.05$).

Table (6): Effect of feeding diets containing different levels of *Nigella sativa L.* seeds on serum proteins.

Experimental Diets	Total protein* g / 100 ml serum	Albumin*	globulins*	glubulin ratio
G_1, negative control	6.66 ± 0.18	3.89 ± 0.10	2.77 ± 0.08	1.40 ± 0.003
G_2, positive control	5.39 ± 0.87	3.14 + 0.51	2.24 ± 0.36	1.40 ± 0.003
G_3, injected rats fed on 1% Nigella Sativa L. Seeds	5.76 ± 0.52	3.36 ± 0.30	2.40 ± 0.22	1.40 ± 0.003
G_4, injected rats fed on 2% Nigella Sativa L. Seeds	5.46 ± 0.61	3.19 ± 0.35	2.28 ± 0.25	1.40 ± 0.003
G_5, injected rats fed on 10% Nigella Sativa L. Seeds	5.73 ± 0.53	3.34 ± 0.31	2.39 ± 0.22	1.40 ± 0.002

* Normal value of serum total protein in rats : 4.48 - 10.20 g/100ml *(Burns and Lannoy, 1966).*

* Normal value of serum albumin in rats : 2.70 - 5.10 g/100ml *(Nomura, et al., 1975).*

* Normal value of serum globulins in rats : 0.39 - 1.60 g/100ml *(Nomura, et al., 1975).*

also enter plasma during the intraplasmatic hydrolysis of the triglyceride of chylomicrons and VLDL.

Supplementation of the diet with 1% *Nigella sativa L.*seeds (G_3) provoked a large rise in total serum cholesterol (48% greater than negative control and -16% lower than positive control) which was evident after 9d. Feeding either 2% or 10% *Nigella sativa L.* seeds has a significant effect (tended to be lower) on serum cholesterol ($p < 0.05$), table 5, when compared with positive control feeding (G_2).

Serum Protein:

When either 1 g or 10 g Nigella sativa / 100 g diet were incorporated into the diets fed to rats for 9d, a significant, reduction in absorption of total protein and albumin was noticed table 6, than in those fed negative diet. Whereas those in rats fed the positive diet (G2) did not differ significantly from those in animals fed 1, 2 and 10g/100g diet Nigella sativa, table 6.

Serum globulin concentrations among groups, tended to be higher than the normal value, ($p<0.05$), table 6.

There are conflicting reports on the changes in the serum proteins in diabetic nephropathy (Lewis, 1955; Schertenleib and Tuller, 1958). They reported that, the clinical material on which the investigations was made varied considerably. The only constant abnormality is a low serum albumin, but a majority has also found raised α-2-globulin levels. Alterations in the different protein fractions have been thought to be significant in the pathogenesis of nephropathy,

Table (7): Effect of feeding diets containing different levels of *Nigella sativa L.* seeds on the activities of serum transaminases (GOT and GPT): serum uric acid and urea of rats.

Experimental Diets	*S. GOT* μ/L	*S. GPT* μ/L	*S. GOT / S. GPT*	*S. Uric acid* mg/dL	*S. Urea** mg/dL
G_1, negative control	22.36±2.14	10.62±1.1	2.13±0.32	26.06±4.34	9.01±1.50
G_2, positive control	45.16 ±2.69	20.00±0.51	2.26±0.11	44.64±3.66	15.43±1.27
G_3, injected rats fed on 1% Nigella Sativa L. Seeds	41.55±4.64	19.65±1.07	2.12±0.29	41.36±2.81	14.29±0.97
G_4, injected rats fed on 2% Nigella Sativa L. Seeds	42.53±3.71	19.74±1.37	2.16±0.15	41.11±4.43	14.20±1.53
G_5, injected rats fed on 10% Nigella Sativa L. Seeds	43.02±4.61	18.97±1.31	2.27±0.25	40.70±4.70	14.07±1.63

* Normal value of serum urea in rats :

8.00 - 27.50 mg/100ml *(Burns and Lannoy, 1966).*

(Ejarque, et al., 1959). The mean serum albumin level in patients who had undergone renal biopsy and had been graded according to the degree of histological change falls progressively with increasing degrees of histological severity of the diffuse lesion but there appears to be no difference in the globulin fractions in the different groups (1.0. 4. g/100ml). The fall in serum albumin is unlikely to be due to proteinuria alone and effective protein synthesis may be responsible (Krahl, 1953).

Serum transaminases (GOT and GPT), serum uric acid and urea:

No significant differences between treatment means on the activities of serum transaminases (GOT and GPT); serum uric and urea, table 7, when injected rats fed diets containing different levels of *Nigella sativa L.* seeds. Serum transaminases (S. GOT and S. GPT); S. Uric acid and S. Urea concentration were insignificantly decreased than positive control rats in all treatment diets group (G3: G4 and G5). Table 7.

Administration of the low level of *Nigella sativa L.* seeds (1%) to alloxanized diabetic rats was characterized by the lowest values of S. G. O. T. than the higher level (10% but this decrease was not significant. The mean values were 41.55±4.64; 42.53±3.71 and 43.02±4.61 μ/L respectively, wherein the results obtained by S. G. P. T. was contrary. Table 7 S. G. P. T. level was increased in both 1% and 2% *Nigella sativa.* This increase may be due to acute liver damage and hepatic dysfunction. On the other hand, high level of *Nigella sativa L.* seeds (10%) fed to alloxanized diabetic rats insignificantly decreased S. Uric acid than the low level (1%) . Tabe 7.

CONCLUSION

1. Nigella sativa L. seeds had no hypoglycemic effect
2. Nigella sativa L. seeds at either 2% or 10% had lower relative liver weights than rats fed all other test diets.
3. Feeding either 2% or 10% Nigella sativa L. seeds had a significant effect (tended to be lower) on serum cholesterol ($p<0.05$).
4. Whilst 1% Nigella sativa L. seeds feeding was accompained by higher serum total lipids concentration. whereas serum triglyceride significantly increased slightly.
5. Serum total protein and albumin in rats fed 1,2 and 10g/100g diet *Nigella sativa* did not differ significantly from those fed positive diet, wherein serum globulin concentrations differed significanlty among groups, tended to be higher than the normal value.
6. Serum transaminases (S. GOT and S. GPT): S. Uric acid and S. urea concentration insignificantly decreased in all treatment diets group

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