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Effects of *Nigella sativa* Seeds and its Oils Fraction on Some Biochemical Parameters in alloxan Diabetic Rats.

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ABSTRACT

The aim of the research study was extended to establish the chemical Composition of Nigella Saliva Seeds and to evaluate the effect of its administration and its oils fraction on serum glucose, total cholesterol, and LDL-Cholesterol of rats suffering from alloxan diabetes.Composition analysis revealed that it contains appreciable quantities of carbohydrates, protein, and fat. Minerals content showed high concentrations of phosphorus (620mg/ 100gm dried wt.) , calcium (202 mg /100gm) and potassium (170mg /100gm). Whilst considerable amount of trace elements such, as iron, Zinc and copper.Characterization of fixed oil enumerated that unsaturated fatty acids linoleic acid and oleic acid are the dominating fraction i.e55.7mg/100gm and 24gm / 100g as Compared to Saturated fatty acids palmitic acid and stearic acid i.e 13g/100g and 2.82g / 100g. Hyperglycemia was induced in the rats by intraperitoneal injection of alloxan at dose of 125mg/ kg body weight. Fifty of alloxan diabatic rats were divided to 5groups, each contain 10 rats of both sex. the 1st group was kept on basal diet without any treatment and considered as control (diabetic)The other four groups were taken a basal diet with 5% Nigella Sativa seeds, 2.5% crude oil, 2.5%fixed oil and volatile oil(3mg TQ\ kg body weight),respectively. Body Wt.gain of the rats did not show any statistical differences by using all the tested diet after 4and 8 weeks (the end of experiment) as compared with the diabetic control. While the food intake was significantly decrease. The levels of serum glucose, total cholesterol and LDL– cholesterol were significantly decreased on diabatic rats after treatment

Keywords: Nigella sativa, fatty acid ,Alloxan diabetes rats

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INTRODUCTION

Nigella Sativa seeds, which is classified in the family of Ranauculacea, has been Shown to contain fixed oil and volatile oil The volatile oil has been shown to contain 18.4%to 24% thymoquinone and 46%monterpenes such as pcymene and a – piene [1]. Medicinal plants play an important role in pharmacology and medicine for many years. Clinical and animal studies have shown that extracts of the Nigella Sative seeds have many therapeutic effects Such as bronchodilation, immunomodilative, antibacterial, hypotensive, antidiabetic, hepatoprotective gastorprotective, antihistaminic, antioxidativeand neuroprotective effects [2].

It has been reported that the beneficial effects of the oil of the Nigella sativa seeds and its active ingredients thymoquinone (TQ), reduce the toxicity induced by the anticancer drug cyclophoshamide through enhancement of antioxidant mediator levels.

It has also been shown that Nigella sativa oil can reduce potent antiviral effects associated with enhanced T-cell responses [3]. Nigella sativa oil has been used for treatment of experimentally induced diabetes in animals based on its combinated hypoglycalmic and immunopotentiating effects that help in ameliorating the impaired immunity and infections associated with diabetes [4].

Nigella sativa produces antiaherogenic effects by decreased significantly serum triglyceride, total and LDL – cholesterol after treatment with 750 mg of powder grain of Nigella sativa enclosed in a capsule two daily for 28 days [5].

The aim of this research is extended to establish the chemical composition of Nigella saliva seeds, fatty acids composition and evaluate the effects of its administration on serum glucose, total cholesterol and LDL- Cholesterol of rats suffering from alloxan diabetes.

MATERIAL AND METHODS

Plant material

- Nigella sativa seeds were supplied by Field crop Research, Institute Agricultural Research center, Ministry of Agriculture. Nigella sativa seeds were cleaned with water and dried in electric oven at 40 °c until, a constent weight was reached .,crushed the seeds using an electric grinder at speed 6 for 2 min. and passed through a 35 mm (42 mesh)sieve then kept on clean container at 4 °c till analysis.
- Volatile oil of Nigella sativa (thymoquinone) was obtained from sigma chemical comp.
- The crude oil was found to be main component of Nigella sativa seeds,and was separated by cold pressing of seeds [6]. The crude oil consisted of fixed oil and volatile oil .
- The fixed oil was obtained using soxhlet extraction of the ground dried seeds for 24hr using petroleum ether (40°c – 60°c) according to procedures of IUPA [7].

Methods

Proximate analysis of dried nigella sativa seeds

Moisture content, total ash, crude protein, crude fiber and fat content were determined in ground dry seeds according to AOAC [8]. Phosphorus was determined spectrophotometrically according to cottenie et al. [9]. Calcium, magnesium, and potassium were determined by flam photometer according cottenie et al. [9]. Zinc, iron, manganese and copper were determined by atomic absorption spectrophotometer [10], The results were expressed on dry weight basis.

Fatty acids composition of ether extract of Nigella sativa oil was determined by gas liquid chromatography (GLC) after hydrolysis and methylation of oil [11].

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Animal experiment

Animals

In current study 60 wisterstrain, albino rats of both sexes weighting 120-160gm were taken from central laboratory of animal house (N.R.C). The animals were kept in individual stainless steel cages under standard laboratory condition. Rats were subjected to controlled condition temperature (25±2 °c) on a 12hr light/dark cycle. The animals were fed with standard diet and water ad libitum.

Induction of hyperglycemia in rats

Hyperglycemia was induced in Fifty rats by intraperitoneal injection of 5% solution of alloxan in saline (125 mg 1 kg) body weight [12]. Before induction of hyperglycemic animals, fasting blood samples were withdrown from retro-orbital vein for estimation the level of glucose. After that rats were then injected with alloxan. After one week of the induction, blood sample was taken for analysis of fasting blood glucose which follows up for 3 weeks to ensure the induction of hyperglycemia.

Experimental design

Fifty of alloxan diabetic rats were divided into 5 groups, each contain 10 rats of both sexs. The 1st group was kept on basal diet without any treatment and considered as control (diabetic). The other four groups were taken Nigella sativa seeds, crude oil, fixed oil and volatile oil respectively as shown in table (3). In addition 10 normal rats were fed basal diet without any treatment as normal control according Reeves [13]. Salt mixture and vitamins mixture were prepared according AOAC [14]. The experiment continued for 8 weeks, during the experiment food intake was daily recorded; body weight was recorded twice a week. Fat soluble vitamins were given orally in a dose of o.l ml/rat/week.

Table 3: The constituents of the diets used in the hyperglycemic experiment (g/100g)

Component of the diet (g)	Diet (1) control diabetic	Diet 2 N.sativa seers	Diet 3 crude oil	Diet 4 fixed oil	Diet 5 volatile oil
Casein	12.5	12.5	12.5	12.5	12.5
Corn starch	69.0	64.0	66.5	66.5	69
Corn oil	10.0	10.0	10.0	10.0	10
Cellulose	4.0	4.0	4.0	4.0	4.0
Salts mixture	3.5	3.5	3.5	3.5	3.5
Vitamins mixture	1.0	1.0	1.0	1.0	1.0
N. salive seeds	-	5.0	-	-	-
Crude oil	-	-	2.5	-	-
Fixed oil	-	-	-	2.5	-
Volatile oil	-	-	-	-	0.003
(thymoquinone)					
Total	100	100	100	100	100

***volatile oil (thymoquinone) was administered orally at a dose level of 3mg/kg body weight**

After 4 weeks of treatment , blood samples were taken to measure the investigated parameters – At the end of the experiment (8 weeks) all rats were sacrificed by decapitation under mild anesthesia (anesthetic ether) and fasting blood sample was withdrown from retro-orbital vein for estimation serum glucose [15], total cholesterol [16], LDL – cholesterol [17].

Statistical analysis of data

The method used for the analysis of the present results are given by Snedecor and Cochran [18].

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RESULTS

Chemical composition of Nigella sativa seeds

Table (1) illustrated proximate analysis of Nigella sativa seeds, it showed that moisture content (5.8%), crude protein (19.8%), total carbohydrates (34.7%) while ash content (4.5%) and fat (35.1%). In additain minerals content are presented in the same table. Data obtained revealed high concentrations of phosphors, calcium and potassium. Trace elements, such as zinc, iron and copper were found in considerable amounts.

Fatty acids composition is presented in table (2). Results indicated that the unsaturated fatty acids linoleic acid and oleic acid are the dominant two fatty acids components of Nigella sativa oil. The saturated fatty acids, palmitic acid was the third fatty acid based on its concentration giving value of 13g/100g dried weight, followed by the stearic acid which constitutes 2.85g/100g dried weight.

Table 1: Chemical analysis of Nigella sativa seeds on dry weight bases

Proximate composition	wt/ 100 gm dry sample
Moisture	5.80
Fat	35 .10
Carbohydrate	34.70
Crud fiber	6.20
Protein (nx6.25)	19.80
Ash	4.20
Minerals	mg/100gm
Potassium	170.00
Calcium	202.00
Magnesium	51.00
Phosphorus	620.00
Zinc	6.10
Iron	10.30
Copper	1.10

Table 2: Fatty acids composition of Nigella sativa L. seeds oil (gm/100g)

Fatty acids	gm/100 gm
Palmitic	13.00
Stearic	2.85
Meristic	0.20
Linoleic	55.70
Oleic	24.60
Linoleic	0.64
Arachidic	0.22
Eicosadienoic	2.70
Total saturated	16.05
Total unsaturated	83.95

Animal Experiments

Biological assessment of different Nigella sativa seeds and its oil fraction deits on hyperglycemic rats:-

Results of the effect of different diets of Nigella sativa seeds and its oil fractions on body weight, and food intake of diabetic rats after 4 and 8 wks are presented in table (4)

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Table 4: Mean body weight gain and food intake for alloxan diabetic rats fed the diets containing Nigella sativa seeds and its oil fractions after 4 and 8 weeks

Groups	Body wt gain (g)		Food intake(g)	
	4 weeks	8 weeks	4 weeks	8 weeks
Control diabetic Mean ± S.E	29.43 ± 9.8	48.86 ± 12.76	431.85 ± 48.27	704.0 ± 47
Diabatic feeding with Nigella sativa seeds Mean ± S.E	22.5 ± 7.11 NS	35.50 ± 7.95 NS	294.63 ± 19.86 < 0.05	545.5 ± 34.3 < 0.05
Crude oil Mean ± S.E	17.88± 5.13 NS	34.38 ± 74 NS	312.88 ± 13.03 < 0.05	615.53 ± 40 < 0.05
Fixed oil Mean ± S.E	28.13 ± 7.85 NS	41.62 ± 8.71 NS	376.25 ± 21.1 NS	723.5 ± 34 NS
Volatile oil Mean ± S.E	22.13 ± 3.58 NS	41.88 ± 5.31 NS	422.0 ± 42.79 NS	709.5 ± 29 NS

NS = not significant at 5% level

Date given in the table revealed that the used diets showed non-significant effects on the body weight gain of the rats after 4 and 8 wks as compared with control (diabetic), Furthermore the highest body weight gain after 8 wks was obtained by using volatile oil diet (41.88) followed by fixed oil (41.62), Nigella sativa seeds (45.5) and crude oil 34.37, but this increase was non- significance. However, volatile oil and fixed oil did not show significant differences in food intake as compared with corresponding control (diabetic) after 4 and 8 wks. Moreover food intake of rats fed Nigella sativa seeds and crude oil diets decreased significantly after 4 and 8 weeks as compared with diabetic control.

Biochemical measurement

Table (5) showed the effect of Nigella sativa seeds and its oil fractions diets on the level of serum glucose, total cholesterol, LDL- cholesterol, in diabetic rats. Serum glucose levels were significantly decreased in the feeding diabetic groups after 4 and 8 wks (p<o. oo5) as compared with the diabetic control until reaching normal ranges.

There were significant decreases in total cholesterol after 8 wks of feeding. The highest decrease was obtained by feeding crude oil diet, following by fixed oil and Nigella sativa seeds. It was observed also that serum LDL-Cholesterol level deceased gradually till 8 weeks, but these decreases were only significant with nigella sativa seeds and the crude oil diets after 8 weeks.

Table 5: levels of serum glucose, total cholesterol and LDL Cholesterol (mg/dl) of experimental diets

Groups	Parameters					
	Gluose"1"		Total cholesterol"2"		LDL- cholesterol"3"	
	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks
Control diabetic Mean ± S.E	130.80 ± 10.5	129.6± 9	89.2 ± 3.3	96.7 ±1.8	17.0 ± 1.1	19.8± 2.3
Diabetic feeding with						
Nigella sativa seeds Mean ± S.E	63.6 ± 2.1 < 0.005	60.4 ± 3.4 < 0.005	69.0 ± 6.9 < 0.005	73.5 ± 6.1 < 0.005	11.5 ± 1.3 NS	11.3 ± 1.4 < 0.05
Crude oil Mean ± S.E	76.40 ± 5.5 < 0.005	71.5 ± 4 < 0.005	76.4 ± 309 < 0.05	74.4 ± 2.6 < 0.05	16.7 ± 1.9 NS	12.1 ± 1.7 < 0.05
Fixed oil Mean ± S.E	67.6 ± 7.8 < 0.005	69.10 ±4.1 < 0.005	77.2 ± 5.5 < 0.005	82.5 ± 4.2 < 0.05	15.3 ± 2.2 NS	14.8 ± 2.4 NS
Volatile oil Mean ± S.E	86.10 ± 8.2 < 0.005	69.4 ± 4.9 < 0.005	76.7 ± 4.5 NS	91.0 ± 2.8 NS	16.9 ± 1.7 NS	17.6 ± 2.6 NS

Control (normal level) Mean ± S.E, "1" 62.70 ± 2.2, "2" 71.70 ± 5.0, "3" 12.5 ± 1.6

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DISCUSSION

In the present study , it was observed that the proximate analysis and minerals composition of Nigella sativa seeds were showed as following crude protein 19.8% , fat 35.1% , moisture 5.8%,ash 4.2 % crude fiber 6.2% and total carbohydrate 34.7% .

Mineral contents of Nigella sativa seeds showed that It consisted mainly of phosphorus salt 620mg/100g dry weight, calcium 202 mg/100g and potassium 170mg/100g.Trace elements such as zinc , iron , and copper were found in considerable amount (6.lmg/100 ,10.3 mg/100g and1. 1g/100gm) respectively . These results are in harmony with some research and slightly different of other. These discrepancy may be due to the geographic and climatic difference of places where seeds are grown or due to the differences in the analytical techniques used.

Fatty acids compositions of the fixed oil extracted from Nigella sativa seeds showed that the major unsaturated fatty acid was linoleic acid (55. 7g /100g) followed by oleic acid (24.6g/100g), and the saturated fatty acids palmitic acid (13.0g/100g) followed by stearic acids (2.85 g/100g) of Nigella sativa in descending order. These result are on the line with Mohamed [19] , who reported that TLC and GLC analysis indicated that Nigella sativa seeds oil contained significant amount of sterols , linoleic (c18:2) , Oleic (c18:1) and palmitic (c16:0) as in most of that common edible oil , are the main fatty acids.

Sultan el al [20] characterized the indigenous that Nigella sativa and its fixed and essential oils and concluded that the Nigella sativa holds nutraceutical potential against various physiological threats owing to its rich phytochemistry especially due to the presence of thymoquinone , tocopherols , etc Bahman et al [21] studied the chemical composition of the extracted fixed oil and volatile of NSL . Seeds grown in I ran by GC and GC/MS and identified eight fatty acids (99.5%) and thirty. two Compounds (86.7%) in the fixed and volatile oil, respectively.

Biological assessments of different diets of Nigella Sativa Seeds and its oil fractions showed non significant effect on body weight gain of diabetic rats, after 4 and 8 wks as compared with control diabetic. Concerning food intake, it was found that fixed oil and volatile oil diets did not induce any Significant effect on food intake after 4 and 8 weeks as compared with diabetic control, while Nigella Saliva Seeds and crude oil showed significant decreases in food intake after 4 and 8 weeks. The non-effect of Nigella sativa seeds and its oil diets may be due to the unpalatable of the experimental diets of diabetic rats. These results given are in good agreement with those reported by Hassanin and Hassan [22].

Results in Table (5) revealed that the Nigella Sativa seeds and its oil fractions diets significantly decrease the fasting serum glucose levels of alloxan diabetic rats comparing to the diabetic control, until reaching within normal range.

Nigella sativa seeds may improve hyperglycemia through different mechanisms. Rachid et al [23] reported that Nigella sativa stimulate B-cell of islets of Langerhans, of endocrine pancreas. Ali (24) reported that the Nigella Saliva enhances partial regenerating and proliferation of B .cells of islet of Langerhans. The finding was coincided with present study as blood glucose lowering effects is confirmed. Another postulated mechanism of lowering of blood glucose is through reduced production of glucose from liver ,a mechanizing called gluconeogensis. Farah et al [25] and le et al [26], gave the opinion that Nigella sativa reduces intestinal absorption of glucose from the lumen of gut . plawasha [27] concluded that the Nigella Sativa mediates its glucose lowering effect through enhancement of peripheral metabolism of glucose , an increase in insulin release and simultaneosly a reduction in glycogen release or may be due to an intestinal reduction of glucose. Furthermore, Nigella sativa seeds oil contains many bioactive constituent such as thymoquinone, P-Cymene, pinene, dithymoquinone and thymohydroquinone [28].

Data concerning the effect of Nigella sativa seed and its oil fractions diets on total cholesterol of alloxan diabetic rats showed significantly decrease levels as compared to diabetic control, but such decrease was non-significant for LDL. Cholesterol by feeding fixed oil diet. Similar results revealed that Nigella sativa either in powder or oil form was shown to reduce significantly total cholesterol and LDL-cholesterol levels , after treatment for 2,4,6 and 8wks compared to the positive control [5] .

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The hypocholesterolemic effect of Nigella sativa seeds many be attributed to the synergistic effect of its different constituent , soluble fiber , sterols , flavonoids and high content of poly unsaturated fatty acids , and might be related to decrease cholesterol absorption and increased bile acid synthesis . It is also may be related to up - regulation of low density lipoprotein receptors and inhabitation of 3-hydroxy . 3-methyl glutaryl coenzyme A reductase [29].

CONCLUSION

From the quality point of view,Nigella sativa seeds and Its fraction oils are a good alternative source of essential fatty acids and these seeds are potentially attractive source of protein, lipid and some common minerals that appear to have a very positive effect on human health. Also have a favourable effect on serum glucose, total cholesterol and LDL. Cholesterol

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