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Comparative study of hypoglycaemic and antioxidant potential of methanolic seed extract and oil of *Nigella sativa* on alloxanized diabetic rabbits

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Abstract: The present study was aimed to investigate the comparative antidiabetic potential of *Nigella sativa* seed extract and oil in an *in vivo* trial using rat animal model. The levels of antioxidants analysed in this study included catalase, vitamin C and bilirubin. NS methanolic extract and its oil were tested for their hypoglycemic effect against alloxanized diabetic rabbits (120mg/kg). The crude methanolic extract and the oil (2.5ml/kg/day) were given orally for 24 days that resulted in a significant reduction in glycaemia, particularly during the first 12 days of treatment (reductions of 58.09% and 73.27%, respectively), whereas the oil treated group normalised the levels of catalase (-69.23%), vitamin C (27.30%), and bilirubin (-51.48%) and the extract treated group normalised the levels of catalase (-65.38), vitamin C (24.15%), and bilirubin (-26.19%) at the end of the trial. The results have shown that the seed oil more significantly normalized the levels of serum catalase, serum ascorbic acid, and total serum bilirubin as compared to the methanolic extract of *Nigella sativa*, so *Nigella sativa* seed oil (NSO) may be used as part of antidiabetic remedies against diabetes and utilized as a nutraceutical.

Keywords: *Nigella sativa*, glycaemia, antioxidant, *Nigella sativa* seed oil, diabetes.

INTRODUCTION

Diabetes mellitus is a disease in which blood glucose level associated with severe complications. The conspicuous cause of ailments and death rate all over the world showed that 2.8% world population suffer diabetes. On the occasion of world diabetes day (Pakistan press release) in 2019, the survey reported that about 17.1% of adult population in Pakistan now living with diabetes (IDF 2019). Recently, the one of the top rated newspaper "The News" According to the International Diabetes Federation (IDF) Diabetes Atlas 10th Edition, which will be published on November 14, 2021, Pakistan is now placed third in the prevalence of diabetes after China and India (The News 2021). Top priorities of world health organization (WHO) to require the immediate solutions of diabetes mellitus as the situation is alarming due to the increased disability and mortality (due to development of diabetic angiopathies) (American Diabetes Association 2021).

The history showed that the plants have been used as a traditional herbal remedy for the welfare of human kinds and also a major perspective of modern medicines. World health organization (WHO) reported that about three fourth populations of limited resource countries depends upon medicinal plants for their first aid treatment with limited access towards the allopathic medicine (WHO 2019; Jamshidi-Kia 2018). Recently, utilization of plants phytomedicine have been improved the various disorders because of not only their easy availability and cheap but also the confidence that natural herbal remedies possessed lesser side effects as compared to self-synthesized medicines (Adib-Hajbaghery and Rafiee 2018). Among the medicinal plants, a very famous and miracle herb *Nigella sativa* (Ranunculaceae) has been used against many ailments since many years ago. The nutritional composition of *Nigella sativa* showed that it contain protein (20.3%), fat (45.4%), moisture (7.1%), ash (7.4%) and the rest of the mass present in the form of carbohydrates. The elemental analysis showed that the potassium, phosphorus, calcium and magnesium chiefly present while sodium, iron, zinc and copper was found with lower levels (Kabir *et al*., 2019). In addition, the various phyto-constituents also found in *Nigella sativa* including alkaloids, saponins, sterols, and essential oil. The black cumin seed oil contain 26-34% fixed oil which constitutes majorly linoleic acid (64.6%) and palmitic acid (20.4%). Along with fixed oil, it also contained essential oil (0.4%-2.5%). The thymoquinone among all the chemical components chief responsible for the broad application against many diseases and used as antidiabetic, antioxidant (Akhtar *et al*., 2020), and hepato-protective agents (Ashraf *et al*., 2020). The *Nigella sativa* seed oil normalized the levels of glucose, lipid contents (TG, TC, LDL-cholesterol and HDL-cholesterol) and uplift the body weight (Akhtar *et al*., 2020). The famous

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bioactive component of *Nigella sativa* such as thymoquinone normalized the blood glucose level, and lipid profile by improving the liver function and oxidative status (Almatroodi *et al*., 2021).

MATERIALS AND METHODS

Collection and extraction of plant materials

The seeds of *Nigella sativa* plants were purchased from local market and authenticated by Department of Botany, University of Sargodha, Sargodha, Pakistan. The plant seeds clean, dry and powdered using electric grinder and macerated in methanol with the ratio (1:10) and shake on orbital shaker at 180 rpm for three days. The macerated material filtered through Whatmann filter paper No. 41. The filtrate store for further study the *Nigella sativa* oil NSO was also purchased from a famous brand available locally, used for trial.

Proximate analysis

The proximate analysis of dried seed powder of *Nigella sativa* was carried out for the estimation of crude fat, crude protein, crude fiber, moisture, ash and nitrogen free extract carbohydrates (NFE) using the procedures given in (AOAC 2000).

The methods are given as under:

The moisture contents were determined by placing the 5g of sample in hot air oven (105°C) for 6 hr. and dried after cooling till the constant weight achieved. The moisture content was calculated as follows

$$\text{Moisture}\,(\%) = \frac{\text{Moisture loss}\,(\text{g})\,\text{x}\,100}{\text{Original wt. of sample}\,(\text{g})}\,\text{x}\,100$$

Moisture loss = Original weight of sample - weight of dried sample

For determining the ash contents, 5g of sample was taken in a pre-weighed crucible and charred on a bunsen burner. Then incineration in muffle furnace at 550°C was done until a constant weight was obtained and finally the ash content was calculated as follows;

$$\text{Ash}\,(\%) = \frac{\text{Wt. of ash}\,(\text{g})}{\text{Wt. of sample}\,(\text{g})}\,X100$$

Using the Kjeldahl's method for determination of the protein contents, sample (500 mg) was mixed with 25mL concentrated sulphuric acid along with 5 g-digestion mixture (K_2SO_4 (90 g) + $CuSO_4$ (7 g) + $FeSO_4$ (3 g)) for 3-4 hours and then heated the device for obtaining the clear/green solution. After dilution with Micro Kjeldahl apparatus, 10 mL of it was distilled with 10mL of 40% NaOH. Titration of the content was carried out using N/10 H_2SO_4 till appearance of golden brown colour The nitrogen contents were estimated by using the formula;

$$\text{Nitrogen}\,(\%) = \frac{\text{Titre of N/10 H2SO4 used x 0.0014 x 250 x 100}}{\text{Wt. of sample x Volume of aliquot sample}}$$

Percentage of total protein in the sample was determined by multiplying the % nitrogen with a factor of 6.25. The crude fat content was determined by placing the thimble (2 g) in soxhlet apparatus and n-Hexane 250mL was added in a 500mL flask. Extraction of fat was executed with petroleum ether for about 4 hours. The contents obtained were placed in a petri dish and dried in an oven. Fat %age was determined as follows;

$$\text{Fat}\,(\%) = \frac{\text{Wt. of fat in sample}\,(\text{g})}{\text{Wt. of sample}\,(\text{g})}\,\text{X}\,100$$

After digestion and filtration, the residues were washed off with hot water to make them acid free. The residues were mixed with NaOH (1.25%, 150mL) and again filtered and washed off with boiling water to get rid of alkali metals and then dried at 70-80°C in an oven for overnight. The residue was overcooked on burner at 550°C for 5-6 hours and then cooled and weighed. The reduction in weight will be equivalent to weight of crude fibre in the sample. The percentage of crude fibre present in sample was calculated as;

$$\text{Crude fiber}\,(\%) = \frac{\text{Weight loss on ignition}\,(\text{g})}{\text{Weight of sample}\,(\text{g})}\,\text{X}\,100$$

The NFE% was calculated as;

NFE (%) = 100 – (moisture % + crude protein % + crude fat % + crude fiber % + ash %)

Determination of mineral

Mineral profile of the plant sample was checked for Na, K, Ca, Mg, Mn, Fe, Cu and Zn through wet digestion method. Dried powder of plant sample (0.8g) was digested on at low temperature (60-70°C) along with HNO_3 (10 mL) for 20 min at hot plate in 100mL conical flasks. Then sample was digested at high temperature (190°C) with $HClO_4$ (60%, 5mL) till the contents in the flask become decolorized. The digested sample was diluted to 100mL by adding distilled water (AOAC 2000). The sample solution was filtered out and the minerals were estimated by atomic absorption spectrophotometer (Model: Varian AA-240). Standard curves were obtained for each mineral by making sample of known strengths. Flame photometer was used for the estimation of sodium and potassium. (Sherwood Flam Photometer 410, Cambridge, UK), minerals were estimated by following protocols described in AOAC (2000)002E

Animals

Fifteen male rabbits (mean weight = 1 kg) were purchased from Department of Pharmacy, University of Sargodha Pakistan and kept in large airy cages at the new animal house of University of Sargodha and were kept in line with "Principals of Laboratory Animal Care" (NIH publication 85-23, revised in 1985). The rabbits were acclimatized for fifteen days prior to start the experiment, and were provided standard diet and water *ad libitum*.

Study protocol

The rabbits were divided into four groups; control, diabetic control, NSSE treated and NSO treated groups, five rabbits per group. Following 14-h fasting, hypoglycaemia was induced by the injection of 10% alloxan (Sigma Chemical Co., St Louis, MO, USA) at a dose of 150 mg/kg (dissolved in isotonic NaCl) (Akhtar *et al*., 2020). Seventy two hours (72 h) after the injection of alloxan, the hyperglycemia was confirmed by the increased level of glucose. The NSSE and NSO administrated orally at a dose of 2.5mL/Kg body weight (Najmi *et al*., 2008) for 24 days. Body weights of all animals were measured on an electric balance on day 1 and at 6-day intervals for the 24 days of the study.

Blood sampling

Determination of plasma glucose levels

On day 1 and at 6-day intervals, all rabbits were fasted for 12 h, and about 2.5mL of blood was taken from the jugular vein using sterile syringes. Blood glucose levels were determined with a glucometer (On Call EZ II, ACON® Laboratories, Inc.). The blood samples were collected in heparin vials to determine antioxidant levels. So the plasma was separated by centrifuging the heparinized blood at 4000 rpm for 9 min, and the plasma was kept at -20 ºC prior to analysis.

Determination of plasma antioxidant levels

Plasma catalase was measured using the method by (Goth 1996). Total bilirubin was determined according to the procedure of **(**Garber 1981), while plasma vitamin C level was estimated with HPLC (Cerhata *et al*., 1994).

STATISTICAL ANALYSIS

Results are presented as mean ± standard deviation. Statistical analysis was carried out with one-way ANOVA analysis and Tukey's post hoc test for multiple comparisons (differences among means), using Statistical Package for Social Sciences (SPSS) software, version 21.0. Differences were considered significant *at* $p<0.05$.

RESULTS

Proximate analysis

The nutritional composition of dried seed sample of plant was done through standard methods and the %age contents was mentioned in table 1.

All values are presented as mean± SD at α=0.05 of three determinations. Different letters (A to E) represent the significant differences within the mean values for a specific parameter

Mineral analysis

Mineral estimation of the sample was done using Flame Atomic Absorption Spectrophotometer. table 2 demonstrated the mineral analysis of seed of *Nigella sativa* for Na, K, Ca, Mg, Mn, Fe, Cu and Zn. The dominant minerals present in this study were found to be potassium (K), magnesium (Mg) sodium (Na) and Zinc (Zn). In *Nigella sativa* the mineral content found were as follow (mg/ Kg); K (1117.4) >Mg (358.8) >Na (122.5) >Zn (84.5). table 2

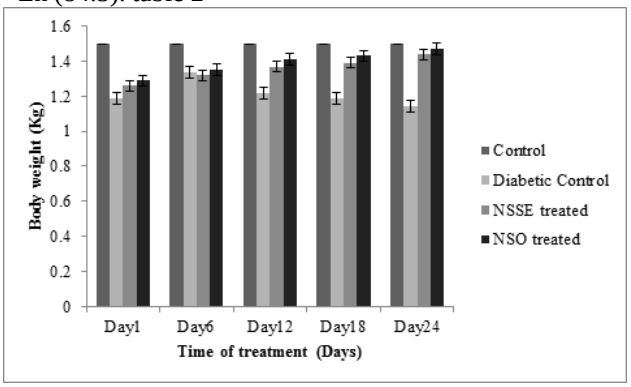

Fig. 1: Effect of NSSE and NSO treatment on body weight Values are expressed as mean + SD (n = 5). $p <$ 0.001, compared to diabetic control; ^{ns} not significant, when compared with diabetic control. The data were analyzed using one-way ANOVA analysis and Tukey's post hoc test. A gradual rise in body weight was seen in alloxan + *NSO* treatment group than NSSE treated group, when compared to the diabetic control as well as NSSE treated groups.

Effect of NSSE and NSO on body weight

The body weight of the animals of all study groups were noted before and after the oral administration of NSSE and NSO. As shown in the figure the significant reduction was found in the mean body weight of the diabetic animals. NSSE and NSO significantly increased the mean body weight at day 24. The most critical effect was observed in case of NSO treated group (fig. 1).

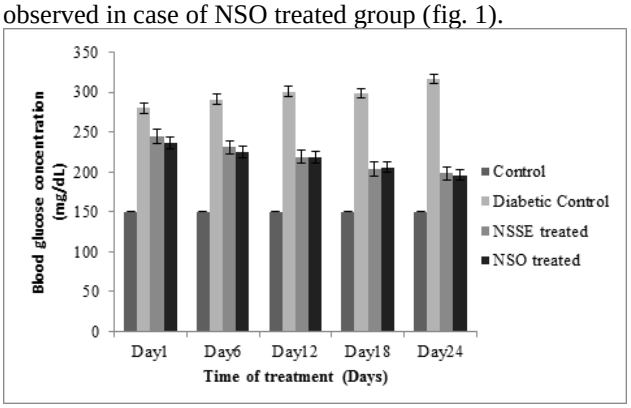

Fig. 2: Effect of NSO and NSSE on blood glucose levels. A gradual decrease in plasma glucose level was observed in alloxan + *NSO* treatment group rather than NSSE treated group, relative to the diabetic control group. Values are mean± SD (n=5), a p <0.001, compared to diabetic control; ^{ns} not significant, relative to diabetic control. The data were analyzed using one-way ANOVA analysis and Tukey's post hoc test.

Table 1: Proximate Analysis

Sr. No.	Parameters	*Nigella sativa*
1	Moisture	6.5424 ± 0.0838^{E}
2	Ash	4.6218 ± 0.049^{C}
3	Fat	8.8145 ± 0.0573^{B}
4	Fiber	71.509 ± 0.2623^{A}
5	Protein	5.1467 ± 0.0660^{B}
6	NFE Carbohydrate	3.2161 ± 0.2306^{D}

Table 2: Mineral Analysis (Flame Atomic Absorption Spectrophotometer) of dried samples; values are given in mg/ kg.

Sr. No.	Samples	Na	K	Ca	Mg	Mn	Fe	Cu	Zn
1.	*Nigella sativa*	122.5	1117.4	0.6	358.8	4.3	2.00	11.1	84.5

From the proximate and mineral analysis, we have found the maximum presence of minerals (K, Mg, Na and Zn) and fibre, protein contents in *Nigella sativa* seed powder

Table 3: Effect of NSSE and NSO on antioxidant parameters

Day	Treatment group	Antioxidant		
		Bilirubin (mg/dL)	*Vitamin C (mg/mL)*	*Catalase (KU/L)*
1	Control	0.76 ± 0.02215	19.61 ± 1.2699	45.8 ± 1.2288
	Diabetic	0.85 ± 0.02935	15.60 ± 1.0569	53.3 ± 1.2748
	NSSE+ diabetic	0.76 ± 0.03708^{ns}(-12.45%)	17.40 ± 1.0114^{ns}(11.68)	48.4 ± 1.342^{ns}(-9.19%)
	NSO+ Diabetic	0.70 ± 0.01591^{a}(-14.63%)	20.60 ± 1.5843^{a}(21.81%)	31.9 ± 1.1726^{a}(-41.3%)
6	Control	0.83 ± 0.05577	19.00 ± 1.2510	48.1 ± 1.5411
	Diabetic	0.88 ± 0.03808	14.98 ± 1.0536	57.6 ± 1.1113
	Alloxan + NSSE	0.81 ± 0.02915^{a}(-3.84%)	16.70 ± 0.9873^{a}(11.48%)	49.4 ± 1.6310^{ns}(-14.23%)
	Alloxan + NSO	0.79 ± 0.02121^{a}(-23.28)	19.31 ± 1.0840^{a}(26.11%)	30.3 ± 1.3210^{a}(-46.81%)
12	Control	0.87 ± 0.05477	18.90 ± 1.2884	44.3 ± 1.5082
	Diabetic	0.57 ± 0.03491	15.50 ± 1.1367	51.1 ± 1.1107
	NSSE+ diabetic	0.87 ± 0.0417^{a}(-52.63%)	18.11 ± 1.1784^{ns}(16.83%)	47.4 ± 1.4311^{ns}(-7.24%)
	NSO + Diabetic	0.86 ± 0.03172^{a}(-68.42%)	20.00 ± 1.0794^{a}(25.17%)	26.5 ± 1.1269^{a}(-48.14%)
18	Control	0.83±0.3131	17.35±1.1341	49.1±1.1231
	Diabetic	0.65±0.2231	12.51±0.9124	59.3±1.0782
	NSSE + diabetic	0.80 ± 0.3134^{a}(-23.07%)	15.52 ± 1.0871^{a}(24.06%)	51.3 ± 1.0021^{a}(-13.49%)
	NSO+ Diabetic	0.81 ± 0.3211^{a}(-24.61)	18.94 ± 1.0266^{a}(41.43%)	48.1 ± 1.1331^{a}(-18.88%)
24	Control	0.85 ± 0.03165	18.65 ± 1.2865	46.40 ± 1.1225
	Diabetic	0.52 ± 0.02739	15.40 ± 1.0770	54.74 ± 1.0559
	NSSE + diabetic	0.86 ± 0.03165^{ns}(-65.38)	19.12 ± 1.0261^{a}(24.15%)	40.40 ± 2.0142^{a}(-26.19%)
	NSO+ Diabetic	0.88 ± 0.03664^{a}(-69.23%)	20.08 ± 1.2510^{a}(27.30%)	27.22 ± 1.3353^{a}(-51.48%)

Effect of NSSE and NSO on blood glucose levels

The glucose level significantly ($p<0.001$) increased after the administration of alloxan to rats when matched with controlled group. The diabetic rats treated with NSSE and NSO, Significant reduction was found in the glucose levels as equated to diabetic rats. Approximately 73.27% reduction was found in NSO treated group as compared to NSSE treated group (58.09%) fig. 2.

Effect of NSSE and NSO on antioxidant attributes

Alloxan administration to rabbits significantly ($p<0.001$) alter the attributes of liver. The levels of bilirubin, catalase was increased while vitamin C level decreased in blood when compared to normal group. Administration of NSSE and NSO to diabetic rabbits, the levels of bilirubin and catalase significantly decreased when compared with diabetic group. While the significant ($p<0.001$) increased was found in the level of vitamin C in blood (table 3).

DISCUSSION

Proximate and mineral analysis of plant seed powders

The finding of the present study showed that protein and fibre contents was found to be maximum in *Nigella sativa* which is comparable to the results reported by (Khalil *et al*., 2021) they also found the maximum content of fibre and protein. The results of mineral analysis showed that the larger content of Potassium (K), Magnesium (Mg) and Sodium (Na) in *Nigella sativa* was comparable to that of (Oubannin *et al*., 2022). Also Copper (Cu) and Sodium

(Na) content in *Nigella sativa* is in close accordance with those of (Oubannin *et al*., 2022) who determined the physicochemical parameters and antioxidant activities seeds and their oils of *Nigella sativa*.

The administration of alloxan to rabbits significantly lowered the mean body weight as compared to the normal group. The decreased in body weight may be due to the result of destruction of beta cells of pancreas and insufficient production of insulin (Woldekidan *et al*., 2021). The finding of the current study is consistent to the results reported by (Abdel-Karim *et al*., 2022), the possible causes of the weight loss include decreased protein synthesis, decreased amino acid intake by tissues, and increased protein breakdown and lipolysis in adipose tissues. Similar observation was also detected in various studies. The administration of NSSE and NSO to diabetic rabbits significantly ($p<0.001$) increased the mean body weight as compared to diabetic group. The results match with the results reported by (Akhtar *et al*., 2020; Chisom *et al*., 2022; El-Gindy *et al*., 2020).

Induction of diabetes altered the level of blood glucose significantly ($p<0.001$) when compared with normal group. The change in the glucose level due the demolition of pancreatic cells which may lead the reduction in the insulin levels, responsible for the increased level of glucose in blood. The alloxan cause the selective necrosis in pancreatic beta cells and induced diabetes by destroying the beta cells of pancreas, resulting in a reduction in endogenous insulin release (Macdonald Ighodaro *et al*., 2017), so the lower concentration of insulin may be due the reduced uptake of glucose by peripheral adipose tissue and skeleton muscles results in the blockage of conversion of fats or lipids in adipose tissue and glycogen in skeleton muscles, responsible for increased level of glucose in blood (Solikhah and Solikhah 2021). The administration of NSSE and NSO significantly lowered the elevated level of glucose when compared with diabetic group. NSO considerably lowered the glucose level as compared to the NSSE. The result of the current studies is matched with the result of (Akhtar *et al*., 2020), who reported that the NSO significantly normalized the blood glucose concentration by the repairing of beta cells of pancreas to secret the sufficient insulin to reduce the glucose in blood. Similarly the same observation was noted by (Sutrisna *et al*., 2022; Akhter *et al*., 2021; Hassan *et al*., 2022), reduction in glucose level may be owing to the restoration of beta cells of pancreas and improving the glucose transportation in tissues (Oza and Kulkarni 2018).

The natural oxidant levels of animal may be responsible to detoxify the adverse effects of reactive oxygen species which lead the destruction of macromolecules, cause a severe illness in animals like diabetes, cardiovascular disorders. Alloxan significantly altered the levels of bilirubin, vitamin C and catalase. After the administration of NSSE and NSO, the levels of antioxidant attributes lowered to normal, especially in case of NSO treated group as compared to NSSE treated group. The findings of the present studies showed that the NSO significantly ($P<0.001$) lowered the elevated levels of bilirubin (-69.23%) and catalase (-51.48%) whereas increased in the level of ascorbic acid was also noted (27.30%). The current results is in agreement to the results reported by (Akhtar *et al*., 2020) who reported that the NSO significantly normalized the levels of bilirubin, ascorbic acid and catalase. Similarly the same observation was noted during the studied trials conducted by (Mohebbati *et al*., 2020; Alghamdi, 2020).

CONCLUSION

The result of the mineral analysis showed that various elements found in *Nigella sativa* seeds were mainly K, Mg, Na, Zn and proximal analysis exhibited that the *Nigella sativa* contained maximum amount of fibers. These elements and proximal composition may be responsible for the synergistic effect of *Nigella sativa*. The NSSE and NSO significantly normalized the antioxidant attributes; bilirubin, ascorbic acid and catalase which may reduce the production of reactive oxygen species responsible for the destruction of pancreatic cell that in turn results in poor production of insulin. NSSE and NSO also reduced the elevated levels of glucose, bilirubin and catalase while increased the level of ascorbic acid. The most significant effects were shown by NSO as compared to NSSE during the entire animal trial. The findings suggest that the *Nigella sativa* extract and oil, used as an herbal remedy against diabetes due to its antioxidant and hypoglycemic activities, may be used as neutraceutical products.

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