



## ***Nigella sativa* ameliorates oxidative stress induced adverse effects in rodent modeling studies: Indices of serum chemistry and hematology**

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### **Abstract**

In this era, various traditional medicinal herbs were researched for their toxicological and safety aspects. The present study focused on using *Nigella sativa* fixed (NSFO) and essential oil (NSEO) to mitigate the adverse consequences of oxidative stress. For the purpose, oxidative stress was induced in Sprague Dawley rats using potassium bromate injection. The effect of NSFO and NSEO on indices of serum chemistry and hematology were studied. The results indicated that potassium bromate imparted drastic changes in serum chemistry and hematology e.g. it increased the blood cholesterol and blood glucose along with decreasing the insulin secretion. Oxidative stress also influenced the hematological attributes negatively but experimental diets were slightly successful in normalizing the values. The experimental diets especially essential oil that contains significant quantities of thymoquinone modulated the adverse consequences of oxidative stress with special reference to hematology and serological attributes. In the nutshell, NSFO and NSEO hold potential to mitigate the oxidative stress, and improved various serological and hematological attributes.

**Keywords:** functional foods; oxidative stress; *N. sativa*; antioxidant; thymoquinone.

**Practical Application:** *N. sativa* is widely used as medicinal plant throughout the world. The study in hand proves that it can be used in the treatment of various diseases and ailments. The industry can use it to prepare different herbal medicine due to its various pharmacological actions.

### **1 Introduction**

Herbs and herbal products have gained immense recognition owing to their health promoting potential. Moreover, scientific innovation and technological advancement provided evidences to support the pivotal linkages between bioactive components and human health (Bjelakovic et al., 2014). Resultantly, functional and nutraceutical foods have emerged as the leading trend in the present decade (Douaire & Norton, 2013). Rich phytochemistry of plants is an important arena of research in the domain of nutrition and pharmacy. Many traditional plants are in use since long to prevent and cure various maladies (Butt & Sultan, 2013). Amongst, *Nigella sativa* (black seed) is quite common in the South Asia, Middle East, Sub-Saharan regions along with some parts of South-East Asia e.g. Malaysia and Indonesia. Compositionally, its bioactive components chiefly reside in its fixed or essential oil e.g. healthy fatty acids, dihomolionolenic acid, tocopherols,

phytosterols, and alkaloids like nigellicine, nigellidine & nigellimine (Cheikh-Rouhou et al., 2007). Furthermore, volatile fractions (essential oil) of *N. sativa* seeds are rich in antioxidants like thymoquinone, *p*-cymene, carvacrol, *t*-anethole, and 4-terpineol; possess varied antioxidant activity (Nickavar et al., 2003).

Across the globe, scientists have attempted to explore aforementioned rich phytochemistry of *N. sativa* to reduce the disease burden. Due to presence of antioxidants, it can be effective in scavenging free radicals and ameliorating the oxidative stress (Sultan et al., 2014). The oxidative stress and its complications could lead to several health disparities including degenerative and neurological disorders. In the last few years, many scientists across the globe investigated the therapeutic potential of black cumin and its bioactive components (Kanter et al., 2009).

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The results from such studies indicated its role in prevention of diabetes mellitus, cardiovascular, and respiratory disorders. It also retrains the free radicals produced in the body that results in adverse consequences to health (Falak & Jamil, 2013).

Previously, safety assessment and toxicological evaluation was conducted (Sultan et al., 2009) in normal Sprague Dawley rats. However, the metabolism differs in normal and diseased conditions, as the metabolism is complex to understand completely. In instant research work, we attempted to elucidate the toxicological aspects related to *N. sativa* fixed and essential oils in oxidative stress conditions. The findings of the present research are helpful in warranting antioxidant potential of *N. sativa* fixed oil (NSFO) and essential oil (NCEO) in improving antioxidant status and decreasing the oxidative damage in potassium bromate oxidative stress.

## 2 Materials and methods

The Barani Agricultural Research Institute (BARI), Chakwal provided us *Nigella sativa* seeds. Sigma-Aldrich Tokyo, Japan and Merck KGaA, Darmstadt, Germany provided us chemical Reagents (analytical & HPLC grade).

### 2.1 Extraction of *Nigella sativa* fixed and essential oils

In the present study, we extracted *N. sativa* fixed oil using Soxtech apparatus. For the purpose, we slurred *N. sativa* seeds with solvent (hexane was used and its ratio was optimized i.e. 1:6). Like fixed oil, we extracted the volatile fraction using hydro-distillation apparatus that was assembled locally in Faisalabad in collaboration with Azeem Scientific Company, Faisalabad, Pakistan.

### 2.2 Induction of oxidative stress and evaluation of test substances

The test substances were *N. sativa* fixed oil @ 4.0% and *N. sativa* essential oil @ 0.30%. The oxidative stress was induced in rats with the peritoneal injection of potassium bromate @ 45 mg/Kg body weight in 0.05M citrate buffer (pH 4.5). The rats were further fed on oxidized corn oil with POV (120 meq/g) to yield chronic oxidative stress.

### 2.3 Test animals and their housing

National Institute of Health (NIH), Islamabad provided infectious free 30 Sprague Dawley rats (Age: 6-7 weeks, weight: 130 ± 10 g). The animals were further divided into three groups of ten rats each and provided them basal-diet (AIN-76A) during the first week to get them acclimatize. The oxidative stress was induced as mentioned in the previous section and three groups received one control & two experimental diets, respectively, for a period of 8 weeks. The compositions of experimental diets include casein (20.0%), corn starch (55.0%), cellulose (10%), corn oil (10.0%), Mineral mixture (4.0%), and vitamin mixture (1.0%). *Nigella sativa* fixed and essential oils were added in the experimental diets @ 4.0% and 0.30%. In case of fixed oil group, corn oil was reduced accordingly and added in the amounts of 6.0%. In the whole study, we made efforts to strictly abide the standard guidelines of Animal Institute of Nutrition (AIN),

USA. Drinking water was provided in bottles, however, feed and water intake were checked daily, while weight of each rat was documented weekly throughout the experimental period. Thrice in the study (0, 28, and 56 days), five rats from each treatment were sacrificed to collect blood for further analysis (Uchida et al., 2001).

### 2.4 Blood lipid profile

For the estimation of blood lipid profile, we used the collected blood samples from each group of rats and parameters estimated were cholesterol, HDL, LDL, and triglycerides. Serum cholesterol level was determined using CHOD-PAP method, HDL Cholesterol Precipitant method for HDL, liquid triglycerides (GPO-PAP) method for total triglycerides (Annoni et al., 1982) and difference method was used to calculate low density lipoproteins (LDL) following the procedure of (Annoni et al., 1982; Assmann, 1979), (Stockbridge et al., 1989), (McNamara et al., 1990).

### 2.5 Serum glucose and insulin levels

For the purpose, GOD-PAP method was followed to determine the glucose concentration of individual rats (Thomas & Labor, 1992). In comparison, ELISA immunoassay technique was employed to observe the values of insulin (Keilacker et al., 1987).

### 2.6 Serum Biochemistry

Total proteins, albumins, globulin and A/G ratio are all indicators for serum proteins profile and these were also measured using commercial kits. Additionally, levels of electrolytes were also assessed to check the efficacy of *N. sativa* fixed and essential oil.

### 2.7 Hematological aspects

Blood samples collected were analyzed for complete blood profile like total red blood cells count, hemoglobin, and hematocrit. Platelets count and erythrocytes sedimentation rates (ESR) were also estimated. Total white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophiles and basophiles were also determined.

### 2.8 Statistical analysis

Regarding the statistical data analysis, values presented in Tables are means ± standard deviation. In order to check the level of significance, we applied analysis of variance (ANOVA) technique and used two factor factorial design and means for diets, study intervals and their interactions were further compared through Duncan's multiple range test (DMRT) following the outlines of (Steel et al., 1997).

## 3 Results

The onset of 21<sup>st</sup> century witnessed mass awareness regarding the diet-health linkages. The purpose of the study was to exploit the rich phytochemistry of *N. sativa* oils against oxidative stress. In order to induce oxidative stress, we used intra-peritoneal injection of potassium bromate @ 45 mg/kg

body weight (Sultan et al., 2012). The results pertaining to the different parameters are discussed herein.

### 3.1 Feed & water intake and body weight

It is obvious from the means that groups of oxidative stressed rats fed on diets containing *N. sativa* fixed and essential oils showed higher feed intakes as compared to significantly lower intake in control group (Figure 1). Likewise, the maximum intake was recorded in NSEO group that was statistically at par NSFO but significantly different than the minimum intake control/placebo group. Likewise, means depicting body weight showed a persistent decline control as compared to increasing trend for body weight experimental diets. Figure 1 represents the feed & water intake and body weight. It depicted the decreasing tendency in feed intake and body weight during study in placebo. However, the experimental diets significantly improved these parameters.

### 3.2 Serum lipid profiles and hypoglycemic perspectives

The lipid profile includes cholesterol, HDL, LDL, and triglycerides. It is evident from the means (Table 1) that *N. sativa* fixed and essential oils based diets were statistically at par in lowering cholesterol significantly with recorded values of  $99.82 \pm 5.33$  and  $102.56 \pm 3.13$  mg/dL, respectively as compared to  $130.82 \pm 11.84$  mg/dL in control group. In control group, LDL contents increased significantly from  $47.44 \pm 3.04$  to  $85.64 \pm 5.33$  mg/dL whereas in fixed and essential oils groups, significant decrease for this trait from  $44.82 \pm 2.89$  to  $31.35 \pm 1.84$  mg/dL and  $45.77 \pm 1.81$  to  $39.24 \pm 1.68$  mg/dL, respectively. Data showing the means for HDL contents indicated the non-significant variations due to diets ranged from  $37.77 \pm 0.65$  to  $39.32 \pm 0.90$  mg/dL. Serum triglycerides increased in oxidative stressed rats fed on control diet from  $108.69 \pm 6.97$  to  $128.35 \pm 7.98$  mg/dL during study. In contrary, fixed and essential oils groups showed significant decrease from  $108.24 \pm 6.97$  to  $86.42 \pm 5.08$  and  $104.43 \pm 4.12$  to  $90.52 \pm 3.87$  mg/dL, respectively. The groups of rats fed on essential oil exhibited significant decrease in blood glucose level from  $113.11 \pm 4.47$  to  $102.40 \pm 4.38$  mg/dL during 56 days study duration. Likewise, *N. sativa* fixed oil decreased the same trait from  $114.13 \pm 2.91$  to  $105.29 \pm 3.57$  mg/dL. However, control group showed marked increase in blood glucose from  $116.22 \pm 6.05$  to  $138.14 \pm 3.58$  mg/dL. *N. sativa* essential oil group showed maximum insulin secretions ( $46.06 \pm 1.50$   $\mu$ U/mL) followed by fixed oil group ( $44.78 \pm 0.90$   $\mu$ U/mL), while minimum level ( $40.30 \pm 1.62$   $\mu$ U/mL) recorded in control. The diets containing fixed and essential oils enhanced the insulin secretions distinctly, however, the same trait dropped significantly in control group.

### 3.3 Indices of serum biochemistry

The means (Table 2) elucidated that diets affected globulin and A/G ratio significantly, while remained non-significant for total protein and albumin contents. However, with the passage of time ratios for albumin contents increased significantly from  $3.54 \pm 0.08$  at start to  $4.20 \pm 0.20$  mg/dL at 56 days of study. The minimum value for globulin contents were recorded in control, while the maximum in NSEO. Diets depicted significant impression on A/G ratio in serum of oxidative stressed rats however, maximum

A/G ratio  $1.44 \pm 0.147$  mg/dL was recorded in D1 (placebo), followed by  $1.20 \pm 0.043$  mg/dL in D2 (*N. sativa* fixed oil) and least  $1.16 \pm 0.067$  mg/dL was recorded in D3 (*N. sativa* essential oil). The levels of potassium were found to be  $4.44 \pm 0.10$ ,  $4.31 \pm 0.07$  and  $5.40 \pm 0.60$  mEq/L in D1 (placebo), D2 (*N. sativa* fixed oil) and D3 (*N. sativa* essential oil), However, maximum levels of bicarbonates  $27.48 \pm 2.91$  mEq/L in NSEO, while the least level  $24.77 \pm 2.66$  mEq/L was established in NSFO group.

### 3.4 Red blood cell indices

Hematological parameters included red blood and white blood cell indices. The results regarding hematology (Table 3) explicated that red blood cell varied non-significantly as a function of experimental diets. However, significant variations

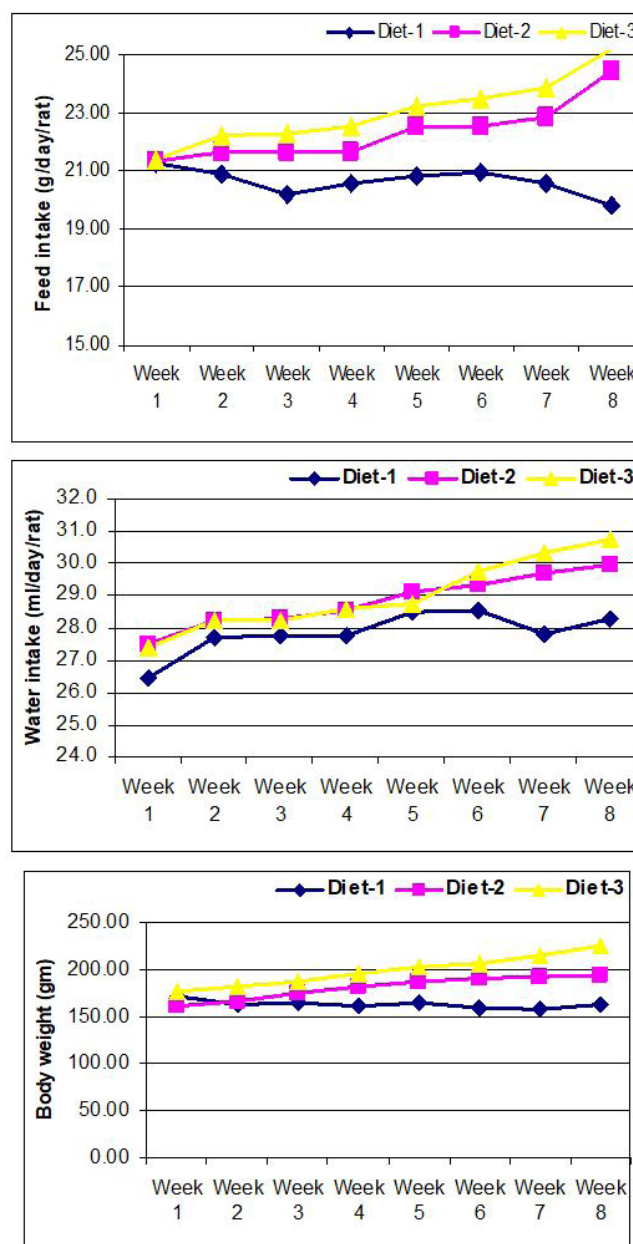


Figure 1. Feed & water intake and body weight of oxidative stressed rats.

**Table 1.** Effects of NSFO and NSEO on indices pertaining to hypercholesterolemia and hyperglycemia organs to body weight ratio in oxidative stressed Sprague dawley rats.

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
Cholesterol (mg/dL)	D <sub>1</sub>	108.15±5.63c	136.26±2.73b	148.06±3.83a	130.82±11.84a
	D <sub>2</sub>	104.05±2.65cd	106.18±4.96cd	89.23±3.03e	99.82±5.33b
	D <sub>3</sub>	105.04±4.15cd	106.30±3.34cd	96.35±4.12de	102.56±3.13b
	Means	105.75±1.24c	116.25±10.01a	111.21±18.54b	
HDL (mg/dL)	D <sub>1</sub>	38.97±2.50	37.58±2.71	36.75±2.29	37.77±0.65
	D <sub>2</sub>	37.58±2.42	39.78±1.86	40.60±2.38	39.32±0.90
	D <sub>3</sub>	38.38±1.52	39.82±1.91	39.01±1.67	39.07±0.42
	Means	38.31±0.40	39.06±0.74	38.79±1.12	
LDL (mg/dL)	D <sub>1</sub>	47.44±3.04c	75.90±5.47b	85.64±5.33a	69.66±11.46a
	D <sub>2</sub>	44.82±2.89c	47.09±2.20c	31.35±1.84d	41.09±4.91b
	D <sub>3</sub>	45.77±1.81c	47.69±2.28c	39.24±1.68cd	44.23±2.56b
	Means	46.01±0.77b	56.89±9.50a	52.07±16.94a	
Triglycerides (mg/dL)	D <sub>1</sub>	108.69±6.97c	113.90±8.21b	128.35±7.98a	116.98±5.88a
	D <sub>2</sub>	108.24±6.97c	96.56±4.51d	86.42±5.08e	97.07±6.30b
	D <sub>3</sub>	104.43±4.12c	93.93±4.49d	90.52±3.87de	96.29±4.19c
	Means	107.12±1.35	101.46±6.26	101.76±13.35	
Glucose (mg/dL)	D <sub>1</sub>	116.22±6.05c	126.31±2.53b	138.14±3.58a	126.89±6.33a
	D <sub>2</sub>	114.13±2.91c	112.24±5.24c	105.29±3.57d	110.55±2.69b
	D <sub>3</sub>	113.11±4.47c	104.35±3.28d	102.40±4.38d	106.62±3.29b
	Means	114.49±0.92	114.30±6.42	115.28±11.46	
Insulin (μU/mL)	D <sub>1</sub>	42.89±2.23de	40.70±0.81e	37.31±0.99f	40.30±1.62c
	D <sub>2</sub>	43.81±1.12cd	43.96±2.05cd	46.58±1.58b	44.78±0.90b
	D <sub>3</sub>	43.92±1.73cd	45.32±1.43bc	48.95±2.09a	46.06±1.50a
	Means	46.54±0.33	43.33±1.37	44.28±3.55	

Means sharing same letters in a column/row do not differ significantly at  $P < 0.05$

in the mean values were established for hematocrit % i.e.  $38.68 \pm 2.11$ ,  $40.94 \pm 1.62$  and  $45.67 \pm 1.37$  in control, NSFO, and NSEO group, respectively. The data pertaining to hemoglobin contents clearly indicated the minimum contents in control as compared to  $14.95 \pm 0.12$  and  $14.90 \pm 0.25$  mg/dL in NSFO, NSEO, respectively. The maximum platelet count of  $4.65 \pm 0.32$  K/ $\mu$ L was recorded in N. sativa essential oil followed by  $4.47 \pm 0.35$  K/ $\mu$ L in N. sativa fixed oil group and the least plate count was recorded ( $3.75 \pm 0.33$  K/ $\mu$ L) in control/placebo, respectively.

### 3.5 White blood cell indices

The results regarding the white blood cell indices (Table 4) showed that diets imparted non-significant effect on white blood cell count

and values were in the range of  $10.10 \pm 0.20$  to  $10.38 \pm 0.36$  K/ $\mu$ L. The mean values for lymphocytes (%) ranged from  $78.53 \pm 4.88$  to  $82.00 \pm 7.30$ %. The maximum neutrophils percentage was  $14.13 \pm 0.40$ % in D<sub>1</sub> (placebo) while the least neutrophils percentage  $13.23 \pm 0.80$ % was in D<sub>3</sub> (N. sativa essential oil). The non-significant variations among the mean values for diets regarding monocytes percentage ranged from  $2.74 \pm 0.06$  to  $2.82 \pm 0.10$ %. Eosinophiles and basophiles varied non-significantly from  $1.26 \pm 0.11$  to  $1.75 \pm 0.15$ % and  $0.64 \pm 0.10$  to  $0.77 \pm 0.10$ , respectively.

### 4 Discussion

Researchers across the globe are more interested in probing the safety assessments and toxicological considerations of traditional



**Table 2.** Effects of NSFO and NSEO on serum protein and serum electrolytes profile in oxidative stressed Sprague dawley rats Serum proteins profile.

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
Total Proteins (mg/dL)	D <sub>1</sub>	7.21±0.462	7.21±0.520	7.74±0.482	7.39±0.177
	D <sub>2</sub>	6.86±0.442	7.26±0.339	7.65±0.449	7.26±0.228
	D <sub>3</sub>	7.31±0.289	7.16±0.343	7.71±0.330	7.39±0.164
	Means	7.13±0.14	7.21±0.03	7.70±0.03	
Albumins (mg/dL)	D <sub>1</sub>	3.70±0.237	4.00±0.288	4.60±0.286	4.10±0.265
	D <sub>2</sub>	3.42±0.220	3.83±0.179	3.96±0.233	3.74±0.119
	D <sub>3</sub>	3.50±0.138	3.62±0.173	4.05±0.173	3.72±0.167
	Means	3.54±0.08c	3.82±0.11b	4.20±0.20a	
Globulins (mg/dL)	D <sub>1</sub>	3.10±0.199	2.80±0.202	2.70±0.168	2.87±0.120c
	D <sub>2</sub>	3.05±0.196	3.02±0.141	3.26±0.191	3.11±0.075b
	D <sub>3</sub>	3.40±0.134	3.06±0.146	3.22±0.138	3.23±0.098a
	Means	3.62±0.11	2.96±0.08	3.06±0.62	
A/G Ratio	D <sub>1</sub>	1.19±0.077	1.43±0.103	1.70±0.106	1.44±0.147a
	D <sub>2</sub>	1.12±0.072	1.27±0.059	1.21±0.071	1.20±0.043b
	D <sub>3</sub>	1.03±0.041	1.18±0.057	1.26±0.054	1.16±0.067b
	Means	1.11±0.05c	1.29±0.07b	1.39±0.16a	
Sodium (mEq/L)	D <sub>1</sub>	133.92±8.59	134.26±9.68	131.02±8.15	133.07±1.03
	D <sub>2</sub>	134.26±8.65	131.68±6.15	134.41±7.89	133.45±0.89
	D <sub>3</sub>	122.21±4.83	130.87±6.26	136.56±5.84	129.88±4.17
	Means	130.13±3.96	132.27±1.02	134.00±1.61	
Potassium (mEq/L)	D <sub>1</sub>	4.60±0.29	4.46±0.32	4.26±0.27	4.44±0.10
	D <sub>2</sub>	4.43±0.29	4.32±0.20	4.19±0.25	4.31±0.07
	D <sub>3</sub>	6.57±0.26	5.06±0.24	4.56±0.20	5.40±0.60
	Means	5.20±0.69	4.61±0.23	4.34±0.11	
Chlorides (mEq/L)	D <sub>1</sub>	131.39±8.42	124.66±8.99	136.49±8.49	130.85±3.43
	D <sub>2</sub>	124.00±7.99	142.73±6.66	142.50±8.37	136.41±6.21
	D <sub>3</sub>	137.76±5.44	135.75±6.50	144.85±6.20	139.45±2.76
	Means	131.05±3.98	134.38±5.26	141.28±2.49	
Bicarbonates (mEq/L)	D <sub>1</sub>	24.00±1.54	27.83±2.01	30.33±1.89	27.39±1.84
	D <sub>2</sub>	23.00±1.48	21.31±0.99	30.01±1.76	24.77±2.66
	D <sub>3</sub>	22.13±0.87	28.16±1.35	32.15±1.38	27.48±2.91
	Means	23.04±0.54	25.77±2.23	30.83±0.67	

D<sub>1</sub> = Control diet; D<sub>2</sub> = Black cumin seed fixed oil; D<sub>3</sub> = Black cumin seed essential oil

medicines. A well-defined research can provide evidences about dietary/health claims associated with some traditional foods or medicines. The addition of *N. sativa* fixed and essential oil increased feed and water intakes as compared to control group. Groups of rats fed on experimental diets gained higher weights

as compared to control. The declining tendency in the body weight during oxidative stress conditions is in well agreement with the findings of (Kanter et al., 2009). These undesirable effects are due to potassium bromate. *N. sativa* fixed and essential oils modulated organ to body weight ratio significantly. According to

**Table 3.** Effects of NSFO and NSEO on red blood cell indices in oxidative stressed Sprague dawley rats in oxidative stressed rats.

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
RBC (M/dL)	D <sub>1</sub>	6.98±0.45	7.87±0.57	6.86±0.43	7.24±0.32
	D <sub>2</sub>	7.02±0.45	6.85±0.32	7.82±0.46	7.23±0.30
	D <sub>3</sub>	7.98±0.32	6.96±0.33	7.14±0.31	7.36±0.31
	Means	7.33±0.33	7.23±0.32	7.27±0.29	
Hematocrit %	D <sub>1</sub>	42.90±2.75	36.44±2.19	36.69±2.28	38.68±2.11
	D <sub>2</sub>	43.50±2.80	37.94±1.77	41.37±2.43	40.94±1.62
	D <sub>3</sub>	43.30±1.71	48.03±2.30	45.67±1.95	45.67±1.37
	Means	43.23±0.62	40.80±3.64	41.24±2.59	
Hemoglobin (mg/dL)	D <sub>1</sub>	13.92±0.89	13.32±0.96	12.71±0.79	13.32±0.35
	D <sub>2</sub>	14.72±0.95	15.00±0.70	15.13±0.89	14.95±0.12
	D <sub>3</sub>	14.71±0.58	14.60±0.70	15.40±0.66	14.90±0.25
	Means	14.45±0.27	14.31±0.51	14.41±0.86	
ESR (mm/Hr)	D <sub>1</sub>	4.48±0.29	4.89±0.35	5.37±0.33	4.91±0.26
	D <sub>2</sub>	4.81±0.31	4.95±0.23	5.90±0.35	5.22±0.34
	D <sub>3</sub>	4.62±0.62	4.55±0.22	5.07±0.22	4.75±0.16
	Means	4.64±0.10	4.80±0.12	5.45±0.24	
Platelet count	D <sub>1</sub>	4.06±0.26	4.10±0.30	3.08±0.19	3.75±0.33
	D <sub>2</sub>	4.15±0.27	4.10±0.19	5.16±0.30	4.47±0.35
	D <sub>3</sub>	4.03±0.16	4.80±0.23	5.11±0.22	4.65±0.32
	Means	4.08±0.04	4.33±0.23	4.45±0.69	

D<sub>1</sub> = Control diet; D<sub>2</sub> = Black cumin seed fixed oil; D<sub>3</sub> = Black cumin seed essential oil

**Table 4.** Effects of NSFO and NSEO on white blood cells in oxidative stressed Sprague dawley rats induces in oxidative stressed rats.

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
WBC (K/ $\mu$ L)	D <sub>1</sub>	10.10±0.19	10.48±0.89	9.76±0.87	10.11±0.21
	D <sub>2</sub>	9.72±0.52	10.19±0.94	10.39±0.77	10.10±0.20
	D <sub>3</sub>	9.66±0.81	10.19±0.90	10.84±0.65	10.38±0.36
	Means	9.83±0.14	10.43±0.13	10.33±0.31	
Lymphocytes (%)	D <sub>1</sub>	78.53±4.88	81.22±6.89	82.00±7.30	80.58±1.05
	D <sub>2</sub>	81.61±4.37	80.19±7.42	79.12±5.87	80.45±0.72
	D <sub>3</sub>	80.14±6.75	79.89±6.80	79.04±4.72	79.69±0.33
	Means	80.09±0.89	80.58±0.38	80.05±0.97	
Neutrophiles (%)	D <sub>1</sub>	15.69±0.97	13.42±1.14	12.15±1.08	13.75±1.04
	D <sub>2</sub>	11.81±0.19	13.32±1.23	14.57±1.08	13.23±0.80
	D <sub>3</sub>	13.40±1.13	14.21±1.21	14.77±0.88	14.13±0.40
	Means	13.19±1.13	13.65±0.28	13.83±0.84	
Monocytes (%)	D <sub>1</sub>	2.74±0.17	2.85±0.24	2.65±0.24	2.75±0.06
	D <sub>2</sub>	2.19±0.14	2.76±0.25	2.82±0.21	2.74±0.06
	D <sub>3</sub>	2.62±0.22	2.89±0.25	2.94±0.62	2.82±0.10
	Means	2.66±0.04	2.83±0.04	2.80±0.08	
Eosinophiles (%)	D <sub>1</sub>	1.34±0.08	1.26±0.11	1.45±0.13	1.35±0.05
	D <sub>2</sub>	1.74±0.09	1.61±0.15	1.73±0.13	1.69±0.04
	D <sub>3</sub>	1.75±0.15	1.68±0.14	1.58±0.09	1.67±0.05
	Means	1.61±0.14	1.52±0.13	1.59±0.08	
Basophiles (%)	D <sub>1</sub>	0.840±0.052	0.580±0.049	0.500±0.045	0.640±0.103
	D <sub>2</sub>	0.920±0.049	0.690±0.064	0.640±0.048	0.750±0.086
	D <sub>3</sub>	0.970±0.082	0.700±0.060	0.640±0.038	0.770±0.101
	Means	0.910±0.038	0.657±0.038	0.593±0.047	

D<sub>1</sub> = Control diet; D<sub>2</sub> = Black cumin seed fixed oil; D<sub>3</sub> = Black cumin seed essential oil

(Ramadan, 2007), antioxidant fractions of *N. sativa* can be employed to improve the antioxidant status of the body and this might be the possible reason for normal ranges of organ to body weight ratio in groups fed on diets containing *N. sativa* fixed and essential oils.

Hypercholesterolemia is an allied consequence of oxidative stress and characterized by elevated levels of cholesterol and triglycerides. In the present research, we observed higher values for cholesterol and triglycerides as compared to normal. In contrary, experimental diets were effective in improving lipid profile as evident from 14.24 and 8.27% decrease in cholesterol in *N. sativa* fixed and essential oils groups, respectively. The experimental diets showed lipid lowering potential and improved antioxidant status. In the present study, blood glucose increased by 18.86% in control, while same trait decreased by 7.75 and 9.47% in NSFO and NSEO groups, respectively. Insulin secretion tends to drop in stress condition by 13.01, while improved by 6.32 and 11.45% in D<sub>2</sub> and D<sub>3</sub> groups, respectively. The findings highlighted the prospects of using appropriate antioxidant rich food to overcome the adverse consequences of oxidative stress including hypercholesterolemia and hyperglycemia (Khalaji et al., 2011).

In the previous study, we observed higher AST, ALT, and ALP activities in oxidative stress (Sultan et al., 2012). The modulation of hematological and serological attributes in oxidative stressed conditions is due to antioxidant perspectives and bioactive components. Such outburst in the activities could further lead to damage to endothelial membranes. Thymoquinone, carvacrol, t-anethol and 4-terpineol are important antioxidants found in *N. sativa* mainly responsible in mitigating oxidative stress. The studies conducted to counteract the CCl<sub>4</sub> damage provided evidence about the protective action of *N. sativa* or its active ingredient thymoquinone, owing to its ability to inhibit lipid peroxidation (Yaman et al., 2010). Oxidative stress disturbs the hematological indices, decreasing the red & white blood cells along with decreasing hemoglobin levels. However, the bioactive molecules present in *N. sativa* holds the potential as antioxidant and thus could be useful for treating ailments characterized with oxidative stress.

## 5 Conclusion

Concluding, *N. sativa* fixed and essential oils exhibited strong antioxidant activity, mitigated the oxidative stress, and improved various serological and hematological attributes. The oxidative stress influenced the hematological attributes negatively but experimental diets were slightly successful to normalize the values. Comprehensive studies (cohort and community based trials) are required to test their effectiveness as nutraceuticals in human subjects.

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