Nigella sativa fixed and essential oil improves antioxidant status through modulation of antioxidant enzymes and immunity

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Abstract: The onset of 21st century witnessed the awareness among the masses regarding the diet-health linkages. The researchers attempted to explore traditional products/plants were in the domain of pharmacy and nutrition focussing on their health benefits. In the present research intervention, we investigate the role of *Nigella sativa* fixed oil (NSFO) and essential oil (NSEO) in improving antioxidant status and modulation of enzymes. The National Institute of Health (NIH) provided us 30 Sprague Dawley rats that were equally placed in three groups. The groups were fed on their respective diets (56 days) two experimental diets i.e. D_2 (NSFO @ 4.0%) and D_3 (NSEO @ 0.30%) and control. The indices pertaining to antioxidant status, antioxidant enzymes, and parameters pertaining to immunity were evaluated at 4 weeks interval. The experimental diets (NSFO@ 4.0% &NSEO@ 0.30%) modulated the activities of antioxidant enzymes i.e., catalase (CAT), superoxide dismutase (SOD), glutathione transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx), positively. Indices of antioxidant status like tocopherols and glutathione were in linear relationship with that of GPx, GR and GST ($P<0.01$). Myeloperoxidase activities were in negative correlation with GST ($P<0.01$) but positive correlation with some other parameters. In the nutshell, the fixed and essential oil of *Nigella sativa* are effective in improving the indices pertaining to antioxidant status, however, the immune boosting potential needs further clarification. However, authors are of the view that there is need to explore the molecular targets of *Nigella sativa* fixed and essential oils. Findings from such studies would be useful to validate this instant study for health promoting potential against diabetes mellitus and cardiovascular disorders.

Keywords: Medicinal plants, functional foods, *Nigella sativa*, essential oil, antioxidant status, antioxidant enzymes.

INTRODUCTION

Over the globe, the pharma foods and dietary supplements are gaining wide range of popularity. Mostly, the bioactive molecules present in traditional medicinal plants are major components of these foods (Butt and Sultan, 2013). The phytochemicals that are present in plants including tocopherols, flavonoids and phenolic compounds are of significance important. These bioactive molecules possess antioxidant activity proven in various *in vitro* and *in vivo* modules (Litescu *et al.,* 2011). The energy yielding metabolic processes in the natural systems results in production of free radicals/reactive oxygen species. Some environmental factors like pollution, pesticide residues, heavy metal toxicity, ingestion of toxicants and direct exposure to ultraviolet radiations are other contributing factors for their over production (Valko *et al.,* 2007). The antioxidants present in the body act as first line of defence to scavenge them but imbalance balance between their production and antioxidants results in oxidative stress (Butt *et al.,* 2009). Overall, oxidative stress results in DNA damage, cardiovascular & neurodegenerative disorders and cancers (Gackowski *et al.,* 2008). In some previous studies, researchers observed the decreased levels of some indigenous enzymes present in human body in these pathological states. The supplementation of antioxidant through dietary modules might help in prevention of oxidative stress and allied ailments. However, the dosage needs to be defined keeping in mind the nutritional requirements and optimal provision through diets that can be helpful in boosting human defence mechanisms (Migliore *et al.,* 2008).

The modern concepts of food synergy and optimum nutrition conceptualized the terms functional and nutraceuticals foods in a comprehensive manner. These foods are somewhat responsible for recent upsurge in nutrition mark*et al*ong with changing consumer's interests towards nature. Globally, several hundred studies explored the health benefits of indigenous plants and foods. Amongst, phytochemical dense plants are important component of herbal and traditional medicines, although they are in use since long for prevention and cure of various maladies. The coupling of nutrition and pharmaceutical together in the form of pharma foods can be effective in reducing the risk of lifestyle related disorders. The mode of action of these foods involve

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multidirectional pathways, however, safety assessment through animal modelling is essential for recommending their further use (Sánchez-Reus *et al.,* 2007).

The medicinal plants hold important position in the flora of Pakistan and various researchers studied the role of plants against various maladies. *Nigella sativa* is used in significant quantities in some Asian countries but limited scientific studies are conducted in the past aimed at exploring its potential role in prevention of ailments. Recently conducted scientific interventions claim its potential use as a functional food and some bioactive compound as nutraceuticals (Butt and Sultan, 2010). The health benefits of *Nigella sativa* seeds are due to presence of bioactive molecules concentrated in its fixed and essential oil. The *Nigella sativa* fixed oil (NSFO) is rich in functional ingredients like tocopherols and phytosterols. In contrary, the *Nigella sativa* essential oil (NSEO) obtained through the process of hydro-distillation contains antioxidants & alkaloids (Sultan *et al.,* 2009a). In the present study, we studied the role of *Nigella sativa* fixed and essential oils (NSFO & NSEO) in improving the antioxidant status and attempted to explore the mechanisms. For the purpose, the levels of hepatic enzymes and some parameters related to immunity were measured. In the nutshell, the upshots of this instant research are quite helpful for the scientists to design future studies in humans related to lifestyle disorders and oxidative damage due to environmental and dietary factors.

MATERIALS AND METHODS

The Barani Agricultural Research Institute, Chakwal provided us with *Nigella sativa* seeds. Chemical Reagents (analytical & HPLC grade) and standards were purchased from Sigma-Aldrich Tokyo, Japan and Merck KGaA, Darmstadt, Germany. National Institute of Health (NIH) Islamabad, Pakistan provided infectious free Sprague dawley rats for the research purpose as per instructions of "Animal Care Committee, NIFSAT-Faisalabad Pakistan".

Extraction of Nigella sativa fixed and essential oils

Following the standard procedures, the seeds of *Nigella sativa* were slurred with hexane (in the ratio of 1:6 using Soxtech apparatus and rotary evaporator was used to remove solvent) to extract the fixed oil. In contrast, *Nigella sativa* essential oil was extracted using locally assembled hydro-distillation apparatus. Earlier to this manuscript, the authors published the results pertaining to the nutritional composition of NSFO and NSEO in Pakistan Journal of Botany (Sultan *et al.,* 2009a), and safety assessment in Food and Chemical Toxicology (Sultan *et al.,* 2009b).

Housing of rats

The National Institute of Health (NIH), Islamabad provided infectious free 30 Sprague Dawley rats that were further divided into three groups of ten rats each. The animals were maintained according to standard guidelines of Animal Institute of Nutrition (AIN), USA i.e. temperature 23±2ºC, relative humidity 55±5%, and 12-hr light-dark cycle. In the first week, the feed of the rats was basal diet in order to acclimatize them to new environment. Later, rats received their respective experimental diets (Composition is mention in table 1) for a period of eight weeks (56 days).

The analytical procedures carried out include parameters measured daily (feed &water intake) and body weight (weekly basis). At 28 & 56 days of feeding trials, five rats from each group were decapitated for blood collection through neck and cardiac puncture (Uchida *et al.,* 2001). The collected blood samples were analyzed for further assays and details are mentioned herein.

Antioxidant status

Glutathione contents were determined following the protocols described by Beutler, (1982). The colour product of GSH + DTNB in the protein free supernatant was measured at 412 nm and expressed as nmol/mg protein. Level of serum α-tocopherol and γ-tocopherol were also measured following the procedures described by Xu and Godber, (1999) through HPLC. Briefly, the oil samples were slurred in hexane as a first step till dissolved completely. A normal phase HPLC column (250mm x 4.6mm, 5.0µm particle size) and mobile phase consisting of isooctane/ethyl acetate (96:4 v/v) was used for the purpose. Total run time and flow rate were 30min and 1.0mL/min, respectively. The detector was set at 290nm excitation wavelength and 400nm emission wavelengths. The column temperature was 35^{o}C. Similarly, the individual standards of isomers of tocopherols were run using the same pattern and curves were obtained and used for calibration in order to determine the amounts of tocopherols in *Nigella sativa* fixed oil. It is for the information of the researchers that the silica column should be reactivated with hexane and isopropanol as mentioned by Panfili *et al*. (2003).

Antioxidant enzymes assays

The estimation of Superoxide dismutase (SOD) activity was based on the reaction between ability of enzyme to inhibit cytochrome 'c' oxidation (Sun *et al.,* 1988) and activity was expressed in IU/mg protein. The decomposition of hydrogen peroxide was measurement of by-products was mainly used for the estimation of catalase (CAT) activity and the units remained the same as IU/mg protein and one IU was equivalent to one µmol H_2O_2 consumed per mg protein per minute (Block *et al.,* 1980).

Glutathione transferase (GST) action was measure using the commercial kits provided by Bioassay Internationals. The reaction includes the rate of formation of conjugate between GSH and 1-chloro-2,4-dinitrobenzene (Beutler *et* *al.,* 1982) and measurement unit used IU/ mg protein. The one IU is equivalent to one μmol of conjugate formed/min/mg-protein. Glutathione peroxidase (GPX) activity was estimated using tertiary butyl hydroperoxide (tbHP) as substrate (Tappel *et al.,* 1978) and the activity was expressed in IU/mg protein and one IU equivalent to one nmol of NADPH oxidized/mg protein in one minutes. Glutathione reductase (GR) activity was assayed by following the oxidation of NADPH. Glutathione reductase (GR) activity was assayed at 37◦C and 340 nm by following the oxidation of NADPH. GR was determined as described by Paglia and Valentine, (1967).

Immunopotentiating aspects

The spectrophotometer was used to estimate the activity of tissue-associated myeloperoxidase (MPO) following the procedures laid down by Hillegas *et al*. (1990). For the purpose, single unit of enzyme activity (IU/mg of proteins) was defined as the amount of the MPO present that caused a change in absorbance measured at 460 nm during the reaction time of three minutes. The indices like xanthine oxidase and nitric oxide are also of significance importance as far as immunopotentiating properties are concerned. The xanthine oxidase was measured using the using diagnostic kits from Cayman Chemicals according following the procedures mentioned by Marcocci *et al*. (1994).

STATISTICAL ANALYSIS

Statistical package i.e. Cohort V-6.1 (Co-Stat Statistical Software, 2003) was used for data analysis. Briefly, values presented in tables are means ± standard deviation. In order to check the level of significance, analysis of variance (ANOVA) technique was applied. The diets (factor A), intervals (factor B) and their interaction (A x B) were used as source of variations. Duncan's multiple range test (DMRt) further clarified the effects of diets in a comprehensive manner.

RESULTS

The onset of 21st century witnessed the awareness among the masses regarding the diet-health linkages. In the present research intervention, we investigate the role of *Nigella sativa* fixed oil (NSFO) and essential oil (NSEO) in improving antioxidant status and modulation of hepatic enzymes. The results pertaining to the different parameters are discussed herein.

Indices of antioxidant status

Glutathione and tocopherols contents are commonly employed for assessment of antioxidant status. Statistical analysis elucidated the momentous effect of diets on α- and γ-tocopherols while non-differential impact on glutathione contents. Glutathione content (table 2) varied non-significantly from 47.33±1.53 to 52.02±0.77mg/L in different diets groups. Although, non-significant difference were observed with reference to diets yet D_3 (*Nigella sativa* essential oil) improved glutathione contents slightly from 50.88±2.67 to 53.49±3.39mg/L.

It is evident from table 2 that α-Tocopherol contents increased in experimental diets as compared to control; maximum (132.85±3.73ng/mL) were recorded in D_2 (*Nigella sativa* fixed oil) and minimum (110.79±4.31ng/mL) in D_1 (control). However, D_3 (*Nigella sativa* essential oil) group with mean α-tocopherol of 127.42±0.68ng/mL was statistically different from other groups. *Nigella sativa* fixed oil was more effective in increasing α-tocopherol contents from 125.43±6.59 to 137.22±6.17ng/mL during experimental rat modelling. γ-Tocopherol (table 2) was recorded highest in D_2 (*Nigella sativa* fixed oil) i.e. 25.28±0.87ng/mLthat was statistically at par with *Nigella sativa* essential oil group with mean value of 25.18±1.09ng/mL. Least content of 22.42±0.54ng/mL was recorded in control.

Table 1: Composition of control and experimental diets

Diet Constituents	D_1	D_2	D_3
Casein (g)	20.0	20.0	20.0
Corn starch (g)	55.0	55.0	55.0
Cellulose	10.0	10.0	10.0
Corn oil (g)	10.0	6.0	10.0
Nigella sativa fixed oil	-	4.0	-
Nigella sativa essential oil	-	-	0.3
Mineral mixture (g)	4.0	4.0	4.0
Vitamin mixture (g)	1.0	1.0	1.0
Total diet weight (g)	100	100	100

D_1 = Control diet; D_2 = *Nigella sativa* seed fixed oil; D_3 = *Nigella sativa* seed essential oil

Antioxidant enzymes

Nigella sativa fixed & essential oils were effective in enhancing activities of glutathione reductase & glutathione transferase significantly as compared to control group (table 3). Activity of glutathione reductase was recorded maximum 33.13±2.150IU/mg protein in D_3 (*Nigella sativa* essential oil) group followed by 31.08±0.757IU/mg protein in D_2 (*Nigella sativa* fixed oil) group while minimum activity of 28.78±0.175IU/mg protein was recorded in D_1 (control). In case of glutathione transferase (table 3), *Nigella sativa* fixed and essential oils resemble statistically with mean activities of 1.28±0.030 and 1.24±0.040IU/mg protein, respectively. However, these groups were significantly different from control that showed minimum activity of 0.98±0.04IU/mg protein. It is evident from the data (table 3) that SOD, CAT, GPx and MPO varied non-significantly due to diets from 12.14±0.894 to 12.85±0.169, 12.23±0.476 to 13.24±0.232 and 115.50±0.671 to 122.97±3.090 IU/mg protein, respectively.

Table 2: Indices of antioxidant status in normal rats

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
Glutathione (mg/L)	D_1	49.77±1.97	47.69±3.03	44.52±2.86	47.33±1.53c
	D_2	50.96±2.56	49.54±2.61	49.51±3.13	50.00±0.48b
	D_3	50.88±2.67	51.68±2.32	53.49±3.39	52.02±0.77a
	Means	50.54±0.90	49.64±0.53	49.17±1.85	
α-tocopherol (ng/mL)	D_1	111.46±4.42	117.89±7.57	103.02±6.56	110.79±4.31c
	D_2	125.43±6.59	135.90±8.62	137.22±6.17	132.85±3.73a
	D_3	126.54±6.36	128.75±8.14	126.97±6.68	127.42±0.68b
	Means	121.14±4.85	127.51±5.24	122.40±10.13	
γ-tocopherol (ng/mL)	D_1	22.59±0.90	21.40±1.36	23.26±1.49	22.42±0.54b
	D_2	23.69±1.24	25.47±1.14	26.67±1.69	25.28±0.87a
	D_3	23.04±1.16	26.58±1.40	25.91±1.64	25.18±1.09a
	Means	23.11±0.32	24.48±1.57	25.28±1.03	

D_1 = Control diet; D_2 = *Nigella sativa* seed fixed oil; D_3 = *Nigella sativa* seed essential oil
Means sharing same letters in a column do not differ significantly at $P < 0.05$

Table 3: Hepatic antioxidants enzymes in normal rats

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
SOD (IU/mg protein)	D_1	12.58±0.498	12.80±0.815	13.16±0.845	12.85±0.169
	D_2	12.32±0.620	12.29±0.647	11.88±0.751	12.16±0.142
	D_3	13.17±0.692	12.89±0.579	10.36±0.657	12.14±0.894
	Means	12.69±0.251	12.66±0.187	11.80±0.809	
Catalase (IU/mg protein)	D_1	13.70±0.543	13.03±0.829	12.98±0.833	13.24±0.232
	D_2	12.97±0.652	11.34±0.597	12.38±0.783	12.23±0.476
	D_3	13.23±0.695	12.79±0.575	12.91±0.819	12.98±0.131
	Means	13.30±0.214	12.39±0.528	12.76±0.189	
Glutathione peroxidase (IU/mg protein)	D_1	116.82±4.629	115.07±7.323	114.62±7.357	115.50±0.671
	D_2	119.39±6.005	122.58±6.451	123.64±7.820	121.87±1.277
	D_3	117.09±6.151	124.25±5.584	127.56±8.088	122.97±3.090
	Means	117.77±0.815	120.63±2.823	121.94±3.831	
Glutathione reductase (IU/mg protein)	D_1	29.10±1.153	28.73±1.828	28.50±1.829	28.78±0.175c
	D_2	29.63±1.490	31.43±1.654	32.18±2.035	31.08±0.757b
	D_3	29.25±1.537	33.45±1.503	36.68±2.325	33.13±2.150a
	Means	29.33±0.16	31.20±1.37	32.45±2.36	
Glutathione transferase (IU/mg protein)	D_1	1.06±0.042	0.92±0.059	0.97±0.062	0.98±0.041b
	D_2	1.25±0.066	1.25±0.079	1.34±0.060	1.28±0.030a
	D_3	1.17±0.059	1.24±0.065	1.31±0.083	1.24±0.040a
	Means	1.16±0.055	1.14±0.127	1.21±0.105	

D_1 = Control diet; D_2 = *Nigella sativa* seed fixed oil; D_3 = *Nigella sativa* seed essential oil
Means sharing same letters in a column do not differ significantly at $P < 0.05$

Indices of Immunity

It is evident from the data (table 4) that MPO varied non-significantly due to diets from 10.70±0.141 to 11.86±0.422IU/mg protein. Means regarding xanthine oxidase activity (table 4) indicated that D_1 (control), D_2 (*Nigella sativa* fixed oil) and D_3 (*Nigella sativa* essential oil) behaved alike with mean activities of 10.57±0.35, 11.11±0.23 and 10.30±0.24IU/mg protein, respectively. It is obvious that there exist non-significant increasing trend for this trait in control and *Nigella sativa* fixed oil groups while declining tendency was observed in *Nigella sativa* essential oil group.

Nitric oxide is important mediator in inflammatory response of immune system. It is evident from means for serum nitric oxide (table 4) that diets varied non-significantly from 10.93±0.46 to 11.48±0.51nmol/dL. However, nitric oxide production was increased non-significantly in D_1 (control) from 10.20±0.40 to 11.79±0.76nmol/dL. In contrary, groups of rats fed on diets containing *Nigella sativa* fixed and essential oils showed declining tendency for this trait from 11.82±0.59 to 10.81±0.68 and 12.12±0.64 to 10.46±0.66nmol/dL, respectively.

DISCUSSION

The diet and health linkages are well defined and many research studies carried out in animal and humans provided evidences that dietary control is best strategy in preventing many ailments e.g. cardiovascular disorders and diabetes mellitus (Butt and Sultan, 2010). The rich phytochemistry of plants is 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. However, the modes of actions are through multidirectional pathways (Butt and Sultan, 2013). *Nigella sativa* is famous in Asian countries owing to its potential role in prevention of ailments also supported from scientific interventions claiming its potential use as a functional food. The health benefits of *Nigella sativa* seeds are attributed to bioactive molecules mainly present in its fixed and essential oils. In this regard, Sultan *et al*. (2009b) carried out the safety assessment of *Nigella sativa* fixed and essential oil (NSFO & NSEO) and reported that both fractions are safe for human consumption. In another study, same fractions were effective in reducing the extent of myocardial necrosis in diabetic rats. In the present project, further efforts were made to study the effect of NSFO and NSEO on hepatic

Table 4: Indices of immune system in normal rats

Parameters	Diets	Study intervals (Days)			Means
		0	28	56	
Myeloperoxidase (IU/mg protein)	D_1	11.12±0.441	12.58±0.801	11.88±0.763	11.86±0.422
	D_2	10.44±0.525	10.92±0.575	10.75±0.680	10.70±0.141
	D_3	11.57±0.608	11.05±0.497	11.34±0.719	11.32±0.150
	Means	11.04±0.328	11.52±0.533	11.32±0.326	
Xanthine oxidase (IU/mg protein)	D_1	10.29±0.41	10.15±0.65	11.26±0.72	10.57±0.35
	D_2	10.65±0.54	11.28±0.59	11.39±0.72	11.11±0.23
	D_3	10.70±0.56	10.34±0.46	9.86±0.63	10.30±0.24
	Means	10.55±0.13	10.59±0.35	10.84±0.49	
Nitric oxide (nmol/dL)	D_1	10.20±0.40	10.79±0.69	11.79±0.76	10.93±0.46
	D_2	11.82±0.59	10.98±0.58	10.81±0.68	11.20±0.31
	D_3	12.12±0.64	11.85±0.53	10.46±0.66	11.48±0.51
	Means	11.38±0.60	11.21±0.33	11.02±0.40	

D_1 = Control diet; D_2 = *Nigella sativa* seed fixed oil; D_3 = *Nigella sativa* seed essential oil

Table 5: Correlation matrix for indices of antioxidant status, hepatic enzymes and immunity

Parameters	GSH	α-Toc	γ-Toc	SOD	CAT	GPx	GR	GST	MPO	XO	NO
GSH	1.00										
α-Toc	0.38^{ns}	1.00									
γ-Toc	0.36^{ns}	0.84**	1.00								
SOD	-0.62*	-0.45^{ns}	-0.60^{ns}	1.00							
CAT	-0.14^{ns}	-0.37^{ns}	-0.66*	0.19^{ns}	1.00						
GPx	0.62*	0.84**	0.90**	-0.76**	-0.45^{ns}	1.00					
GR	0.68*	0.77**	0.82**	-0.81**	-0.29^{ns}	0.95**	1.00				
GST	0.63*	0.91**	0.78**	-0.42^{ns}	-0.42^{ns}	0.84**	0.68*	1.00			
MPO	-0.68*	-0.64*	-0.51^{ns}	0.29^{ns}	0.30^{ns}	-0.54^{ns}	-0.32^{ns}	-0.81**	1.00		
XO	-0.29^{ns}	0.15^{ns}	0.29^{ns}	0.30^{ns}	-0.52^{ns}	-0.01^{ns}	-0.20^{ns}	0.21^{ns}	-0.35^{ns}	1.00	
NO	-0.51^{ns}	0.32^{ns}	0.10^{ns}	0.49^{ns}	-0.08^{ns}	-0.08^{ns}	-0.13^{ns}	0.22^{ns}	0.01^{ns}	0.48^{ns}	1.00

GSH = glutathione; α-Toc = α-Tocopherol; γ-Toc = γ-Tocopherol; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; GR = glutathione reductase; GST = glutathione transferase; MPO = myeloperoxidase; XO = xanthine oxidase; NO = nitric oxide enzymes, antioxidant status and immunopotentiating potential of *Nigella sativa* in normal Sprague dawley rats. The protocols followed in the present research were similar to studies conducted by Singh *et al.* (2002) and Morita *et al*. (2008).

Improvement in antioxidant status of the body certainly assures the normal functioning of the human body (Valko *et al.,* 2007; Sultan *et al.,* 2012). Similar types of studies exploring the role of natural products against oxidative stress were conducted by Celik *et al.,* (2006); reported that plant rich in bioactive molecules may be employed to strengthen antioxidant status of the body that are in agreement with instant findings (Ahmad *et al.,* 2013). *Nigella sativa* fixed oil is rich in tocopherols whereas thymoquinone, carvacrol, thymol, cymene, t-anethole and 4-terpineol are the major antioxidants of *Nigella sativa* essential oil (Wajs *et al.,* 2008; Sultan *et al.,* 2009a). Glutathione and tocopherols are important free radical scavengers present within the body and their role in ameliorating oxidative stress has been highlighted in number of research investigation. In one such study, Casalino *et al.,* (2006) reported that glutathione actively involved in enzymes catalyzed oxido-reduction and its amount determines the antioxidant state of the body. Similarly, Nemmiche *et al.,* (2007) claimed that α-tocopherol supplementation mitigate the adverse consequences of oxidants like cadmium and carbon tetrachloride. Moreover, phytochemicals rich sources are promising candidates for improving antioxidant status (Valko *et al.,* 2007). Antioxidant potential of experimental diets is certainly due to these functional ingredients. Correlation between antioxidant potential of *Nigella sativa* fixed and essential oils and their bioactive molecules has been well defined. The glutathione was positively correlated with hepatic enzymes like GPx, GR and GST, while it was negatively linked with MPO (table 5). The reduction in indices of antioxidant status in control is due to continuous process of aging that depends upon free radical generation. It can be stated generally that antioxidant status reduces with the process of aging (Nakamura and Omaye 2004; Prior *et al.,* 2007).

Process of aging can be reduced with adequate intake of antioxidants. Rich phytochemistry of *Nigella sativa* fixed and essential oils may helpful in this regard (Butt and Sultan, 2010) as evident from the present investigation. According to Celik *et al.,* (2006), balanced activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione transferase (GST) are known to serve as protective elements to eliminate reactive free radicals. In the present study, the activities of these enzymes are within their normal ranges, however, slight improvement in glutathione reductase and transferase were observed. The SOD was recorded to be linked negatively with the same enzymes (table 5). Results of the present plan are in corroboration with the findings of Khan and Sultana, (2003). The findings are correlated with the present investigation that *Nigella sativa* fixed & essential oils are rich in bioactive molecules and their *in vivo* antioxidant activities are well reflected like reduced oxidative damage, improved antioxidant status, and modulation of hepatic antioxidant enzymes.

CONCLUSION

Conclusive approach drawn from this section of efficacy study is, *Nigella sativa* fixed and essential oils hold potential to improve the antioxidant status significantly, whilst, modulating antioxidant enzymes status in normal rats. Experimental diets modulated enzymes and immune system. Although, correlation matrix linked indices of immunity and antioxidant status positively but still the issue demands further studies to determine the molecular perspectives of the health promoting potential of *Nigella sativa*. Accordingly, there is need to conduct further trials in similar type of rats modelling after inducing oxidative stress to validate the findings.

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