



Research Article

The protective effect of *Nigella sativa* oil extract against neurotoxicity induced by Valproic acid

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Abstract: *Nigella sativa* (NS), commonly known as black cumin, has been used for medicinal purposes. Traditionally the seeds and its oil are used in several diseases. The greatest part of the remedial properties of this plant is due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. The current study performed to evaluate the effect of *Nigella sativa* oil (NSO) extract on the neurotoxic and hepatotoxic potentials from valproic acid (VPA) administration. Also we summarize recent findings emphasizing the role of main neurotoxic and hepatotoxic markers and oxidative stress in study's case. Neurotoxicity was induced by VPA at dose of (500 mg/kg b.wt) by gastric intubation daily for 30 day. These rats received NSO extract was given orally at dose of (0.5 ml/kg b.wt) daily for 30 days after VPA administration. The current results revealed that NSO extract treatment ameliorated significantly the elevated levels of the neurotoxic and hepatotoxic biomarkers which elevated as a result to VPA administration. Moreover, NSO extract treatment ameliorated the enzymatic antioxidant, brain and liver catalase (CAT) activity and the non-enzymatic antioxidant, brain and liver glutathione (GSH) and lipid peroxidation (LPO) concentrations.

Keywords: Neurotoxicity, *Nigella sativa* (NS), Valproic acid (VPA).

Introduction

Neurotoxicity has been defined as “any adverse effect on the chemistry, structure or function of the nervous system, during development or at maturity, induced by chemical or physical influences” (Costa, 1998). An adverse effect is “any treatment related change which interferes with normal function and compromises adaptation to the environment” (ECETOC, 1992). Thus, most morphological changes such as neuronopathy (a loss of neurons), axonopathy (a degeneration of the neuronal axon), myelinopathy (a loss of the glial cells surrounding the axon), or other gliopathies, would be considered adverse, even if structural and/or functional changes were mild or transitory. In addition, neurochemical changes, also in the absence of structural damage, should also be considered adverse, even if they are transient and reversible, as they bring about dysfunction (Giordano and Costa, 2012). Neurotoxicity can also happen as a result of indirect effects, such as harm to hepatic or cardiovascular structures, or because of interference with the endocrine systems. Some chemicals may have several manners of action and may influence the nervous system both directly and indirectly (Crofton, 2008).

Valproic acid (VPA) is a branched chain carboxylic acid (2-propylpentanoic acid or di-n-propylacetic acid), with a chemical structure very resemble to that of short chain fatty acids (Johannessen and Johannessen, 2003). VPA is a drug utilized for treatment of epilepsy, schizoaffective and bipolar disorder. It is also most desirable known as a major antiepileptic drug for generalized and partial seizures in adults and children. Despite its wide spectrum usage, valproate is also well known for its idiopathic deadly side effects such as hepatotoxicity and

teratogenicity (Tan *et al.*, 2016), pancreatitis, bone marrow suppression, polycystic ovary syndrome, and VPA-induced hyperammonemic encephalopathy (VHE) (Larsen and Østergaard, 2014) [leading to neurotoxicity and irreversible brain damage (Priester *et al.*, 2009)].

Nigella sativa (NS), usually known as black cumin, belongs to the botanical family of Ranunculaceae which is extensively farmed in the Mediterranean region (Noor *et al.*, 2012). NS has been widely carried out researches for its biological activities and restorative potential and illustrated to have a wide range of activities such as diuretic, antihypertensive, anti-diabetic, anti-cancer, immune-modulatory, antimicrobial, anthelmintic, spasmolytic, bronchodilator, anti-inflammatory, anti-tussive, gastroprotective, hepato-protective, neuroprotective, renal protective and anti-oxidant properties. In traditional medicine, the seeds of NS are generally used as a part of the therapy of various illnesses like obesity, back pain, hypertension and gastrointestinal problems, asthma, cardiac diseases, diarrhea, rheumatoid arthritis and skin disorders (Beheshti *et al.*, 2016). The best part of the corrective properties of this plant is due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil (Darakhshan *et al.*, 2015). TQ reported to demonstrate the anticonvulsant activity (Hosseinzadeh *et al.*, 2005) and also show to display neuroprotective effects (El-Dakhakhny *et al.*, 2002).

Materials and Methods

Preparation of Plant Extract

NS seeds were purchased from a local herbalist in Egypt. The seeds were botanically authenticated by

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specialist of plant taxonomy (faculty of science, Beni-suef University, botany department). The seeds of NS were powdered in a mixer. 20 g of the powdered seeds were added to 400 ml of distilled water and extraction was carried out by steam distillation. The distillation process was resumed until about 200 ml of distillate was accumulated. The distillate was extracted three times with chloroform. Moisture was eliminated by anhydrous sodium sulphate and the resultant extract was evaporated using a 40° C water bath leading to the appearance of the volatile oil. Five hundred milligrams of the volatile oil were dissolved in 1 ml of dimethyl sulphoxide (DMSO) then 9 ml of normal saline was added to yield a concentration of 50 mg volatile oil per 1 ml solution (Fararh *et al.*, 2002). The volatile oil has been shown to include (18.4-24 %) TQ and a total of 46 % of many monoterpenes such as p-cymene and pinene (El-Tahir *et al.*, 1993). El-Tahir reported that, the principle component isolated from NS is TQ.

Experimental animals:

Male albino rats weighing between 100-120 g were used as experimental animals in the present investigation. They were obtained from the Animal House of Abou Rawash, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at normal atmospheric temperature (25±5°C) as well as under good ventilation and received water and standard balanced diet *ad libitum*. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals of Canadian Council on Animal Care (Canadian Council on Animal Care [CCAC], 1993). All attempts were done to reduce the number of used animals and their suffering.

Drugs:

Sodium valproate (VPA) (convulex® produced by Gerot Pharmazeutika Vienna, Austria) was purchased from El-Borg pharmacy (Beni-suef, Egypt) in syrup form. Each 1 ml Convulex syrup contains 300 mg sodium Valproate.

Doses and Treatment:

The dose of sodium valproate used in this study was 500 mg/kg b.wt (Niaraki *et al.*, 2013). This dose was given orally by gastric intubation daily for 30 day. The chosen dose of *Nigella sativa* oil (NSO) extract was 0.5 ml/kg b.wt (Awadalla, 2012). This dose was given orally by gastric intubation daily for 30 days.

Experimental Design:

Animals were divided into four groups comprising six animals in each group designed as the following:

Group (1): was regarded as control group: received distilled water.

Group (2): administered sodium valproate (VPA) orally at dose of (500 mg/kg b.wt) (Niaraki *et al.*, 2013) daily for 30 days.

Group (3): administered *Nigella sativa* oil (NSO) extract was given orally at dose of (0.5 ml/kg b.wt) (Awadalla, 2012) daily for 30 days.

Group (4): rats of this group were administered sodium valproate (VPA) orally at dose of (500 mg/kg b.wt) (Niaraki *et al.*, 2013) daily for 30 days, then treated with *Nigella sativa* oil (NSO) extract was given orally at dose of (0.5 ml/kg b.wt) (Awadalla, 2012) daily for 30 days.

At the end of periods, the six animals of each group were fasted for 12 hours and then sacrificed under mild diethyl ether anesthesia. Blood from each rat was collected from jugular vein in a centrifuge tube and left to clot at room temperature for 45 minutes. Sera were separated by centrifugation at 3000 r.p.m. at 30°C for 15 minutes and kept frozen at -30°C pending biochemical analyses. Brain and liver from each animal was rapidly excised after dissection. 0.5 g was homogenized in 5ml 0.9% sterilized NaCl (10% w/v) using teflon homogenizer (Glas-Col, Terre Haute, USA) for monoamines content in brain tissue and antioxidants defense system assay.

Biochemical analyses:

Butyryl cholinesterase (BChE) activity was determined in serum according to the colorimetric kinetic method of (Knedel and Bottger, 1967) using kits obtained from Bio Diagnostic Company, Egypt. The determination of serotonin (5-hydroxytryptamine (5-HT)), norepinephrine (NE), epinephrine (EP) and dopamine (DA) content in brain tissue homogenate was carried out according to (Ciarolone, 1978). Ammonia (NH₄⁺) concentration was determined in serum according to the colorimetric method of (Konitzer and Voigt, 1963) using kits obtained from Bio Diagnostic Company, Egypt. Urea concentration was determined in serum according to the Urease-modified Berthelot reaction (Young, 2001), using the reagent kits purchased from Diamond Diagnostics, Germany. Aspartate aminotransferase (AST) activity in serum was determined according to the method of (Murray, 1984a) using reagent kits purchased from Spinreact Company, Spin. Alanine aminotransferase (ALT) activity in serum was determined according to the method of (Murray, 1984b) using reagent kits purchased from Spinreact Company, Spin. Total protein in serum was measured according to the colorimetric method of (Young, 2001), using kits obtained from Diamond Diagnostics Company, Germany. Albumin in serum was measured by coloremtric method according to (Gendler, 1984) using kits obtained from Spinreact Company, Spin. Globulin in serum was measured and calculated according to (Doumas *et al.*, 1971). Catalase (CAT) activity in brain and liver tissues was assayed according to method suggested by (Aebi, 1984). Glutathione (GSH) content in brain and liver homogenate was determined according to the method of (Beutler *et al.*, 1963) with some modifications. Lipid peroxidation (LPO) concentration in brain and liver tissues was determined by the method of (Ohkawa *et al.*, 1979).

Statistical analysis:

The data were analyzed on the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Washington Seattle, USA). Using the one-way analysis of variance (ANOVA) Results were expressed as mean ± standard error (SE) and values of P>0.05 were considered non-significantly different, while those of P<0.05, P<0.01 and P<0.001 were considered significantly, highly and very highly significantly different, respectively.

Results

The administered VPA to rats showed a significant increase in activity of serum BChE enzyme when compared with normal rats. Rats-administered VPA treated with NSO extract exhibited an amelioration in

BChE enzyme activity, recording a significant increase as compared to the normal rats (table1 and figure 1).

Table 1: The effect of NSO extract on serum BChE activity of VPA-administered albino rats

Group	Parameter	BChE (U/L)
Normal		42.79 ± 2.17 ^c
VPA		79.42 ± 4.99 ^a
NSO		39.56 ± 2.80 ^c
VPA + NSO		65.10 ± 1.25 ^b
LSD		3.95

- Data are expressed as Mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
- F-probability: p<0.001.

In table 2 and figures 2, 3, 4 and 5, the rats-administered VPA exhibited a significant increase in the homogenate serotonin (5-hydroxytryptamine (5-HT)), norepinephrine (NE), epinephrine (EP) and dopamine (DA) concentrations as compared with normal rats. The rats-administered VPA treated with NSO extract exhibited an amelioration in homogenate NE, EP and DA concentrations, recording a significant increase as compared to the normal rats, but exhibited a non-significant with normal rats but ameliorations occurred in brain homogenate 5-HT concentration.

The rats-administered VPA exhibited a significant increase in serum NH₄⁺, urea concentrations, AST and ALT activities and exhibited a significant decrease in the

serum albumin, but exhibited a non-significant change in both serum total protein and globulin concentrations when compared with normal rats. Rats-administered VPA treated with NSO extract showed exhibited a significant increase with normal rats with an amelioration in serum urea concentration, AST and ALT activities, but a non-significant change as compared to the normal rats with ameliorations noticed in serum NH₄⁺, albumin and globulin concentrations, but no treatment noticed in serum total protein concentration (table 3 and figures 6, 7, 8, 9, 10, 11 and 12).

Brain CAT activity and GSH content were significantly decreased, on the other hand, brain LPO concentration was significantly increased in VPA administered rats. (table 4 and figures 13, 14 and 15). The treatment of these animals with rats with NSO produced a non-significant change in CAT activity, GSH content and LPO concentration but exhibited an amelioration noticed in CAT activity, GSH content and LPO concentration.

Liver CAT activity and LPO concentration was significantly decreased and significantly increased respectively, as a result of VPA administration, while GSH level exhibited a non-significant effect. The treatment of VPA-administered animals exhibited a non-significant change in CAT activity, GSH content and LPO concentration with an amelioration noticed in CAT activity and LPO concentration but no treatment noticed in GSH content (table 5 and figures 16, 17 and 18). NSO extract-administrations exhibited non-significant changes in all parameters as compared to the normal rats.

Table 2. The effect of NSO extract on monoamines concentrations in brain tissue homogenate of VPA-administered albino rats

Group	Parameter	5-HT (µg/g)	NE (ng/g)	EP (ng/g)	DA (ng/g)
Normal		3.62 ± 0.29 ^b	721.53 ± 9.52 ^c	45.70 ± 1.91 ^c	32.93 ± 1.65 ^c
VPA		5.83 ± 0.50 ^a	921.96 ± 21.80 ^a	79.30 ± 6.21 ^a	57.96 ± 3.06 ^a
NSO		3.43 ± 0.33 ^b	736.88 ± 7.50 ^c	46.57 ± 1.96 ^c	35.50 ± 1.60 ^c
VPA + NSO		4.05 ± 0.02 ^b	815.83 ± 46.26 ^b	63.43 ± 2.53 ^b	43.33 ± 2.11 ^b
LSD		0.40	31.83	4.44	3.06

- Data are expressed as Mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
- F-probability: p<0.001.

Table 3. The effect of NSO extract on serum liver functions parameters of VPA -administered albino rats

Group	Parameter	NH ₄ ⁺ (µg/dl)	Urea (mg/dl)	AST (U/L)	ALT (U/L)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Normal		339.37 ± 61.22 ^b	48.89 ± 2.69 ^b	32.48 ± 1.10 ^c	11.92 ± 1.45 ^d	5.48 ± 0.26 ^a	4.63 ± 0.25 ^a	0.85 ± 0.12 ^a
VPA		570.77 ± 80.45 ^a	67.77 ± 3.08 ^a	97.01 ± 8.88 ^a	42.22 ± 8.71 ^a	4.65 ± 0.22 ^a	3.64 ± 0.24 ^b	1.01 ± 0.24 ^a
NSO		345.63 ± 24.98 ^b	47.45 ± 2.27 ^b	47.52 ± 3.05 ^{bc}	20.11 ± 0.95 ^{bcd}	5.21 ± 0.46 ^a	4.47 ± 0.21 ^{ab}	0.74 ± 0.14 ^a
VPA + NSO		456.38 ± 30.01 ^{ab}	58.46 ± 4.40 ^a	54.74 ± 7.29 ^b	33.54 ± 1.16 ^{abc}	4.97 ± 0.23 ^a	4.09 ± 0.19 ^{ab}	0.88 ± 0.21 ^a
LSD		66.71	4.52	8.63	5.93	0.44	0.31	0.38

- Data are expressed as Mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
- F-probability: p<0.001.

Table 4. The effect of NSO extract on antioxidant defense system and oxidative stress of brain tissue homogenate of VPA - administered rats

Group	Parameter	CAT (U/g)	GSH (mg/g.tissue)	LPO (nmol/g.tissue)
Normal		4.77 ± 0.46 ^a	33.11 ± 2.37 ^a	30.19 ± 3.13 ^b
VPA		2.58 ± 0.04 ^b	17.74 ± 2.14 ^b	51.72 ± 7.37 ^a
NSO		4.28 ± 0.41 ^a	31.55 ± 3.35 ^a	35.36 ± 1.77 ^b
VPA+ NSO		3.86 ± 0.29 ^{ab}	26.46 ± 2.29 ^{ab}	41.52 ± 2.42 ^{ab}
LSD		0.48	3.84	5.09

- Data are expressed as Mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
- F-probability: p<0.001.

Table 5. The effect of NSO extract on antioxidant defense system and oxidative stress of liver tissue homogenate of VPA-administered rats

Group	Parameter	CAT (U/g)	GSH (mg/g.tissue)	LPO (nmol/g.tissue)
Normal		14.56 ± 1.54 ^a	26.96 ± 4.01 ^a	22.50 ± 0.25 ^b
VPA		7.49 ± 0.91 ^b	20.89 ± 3.08 ^a	39.95 ± 1.55 ^a
NSO		13.93 ± 0.91 ^a	24.84 ± 1.31 ^a	24.15 ± 3.40 ^b
VPA + NSO		10.76 ± 1.01 ^{ab}	22.09 ± 2.37 ^a	28.25 ± 3.61 ^b
LSD		1.50	4.02	3.21

- Data are expressed as Mean ± standard error.
- Number of animals in each group is six.
- Means, shared only superscript symbol (s) are not significantly different.
- F-probability: p<0.001.

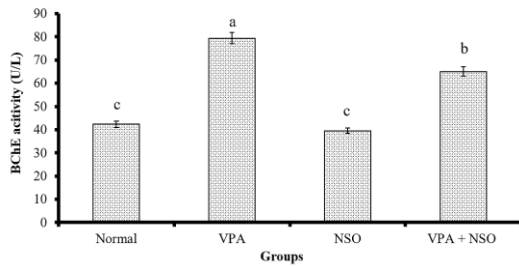


Fig. 1: The effect of NSO extract on serum BCHE activity of VPA-administered albino rats.

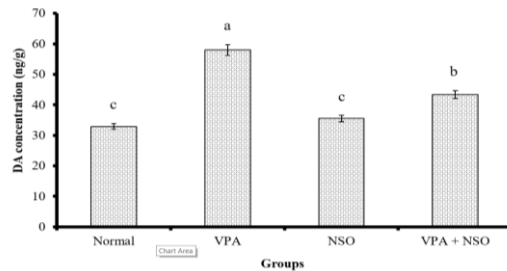


Fig. 5: The effect of NSO extract on homogenate DA concentration of VPA-administered albino rats.

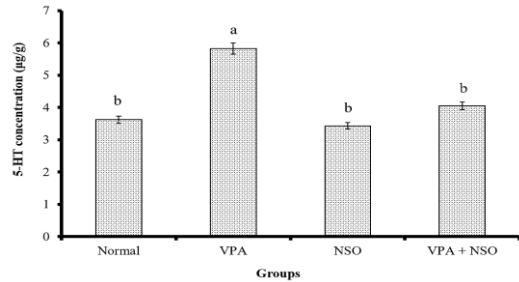


Fig. 2: The effect of NSO extract on homogenate 5-HT concentration of VPA-administered albino rats.

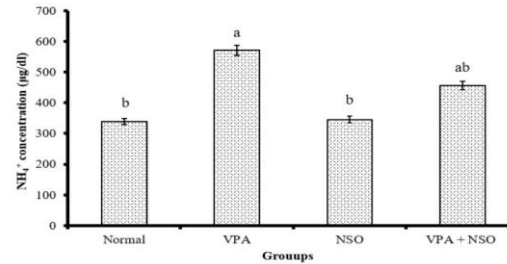


Fig. 6: The effect of NSO extract on serum NH4+ concentration of VPA-administered albino rats.

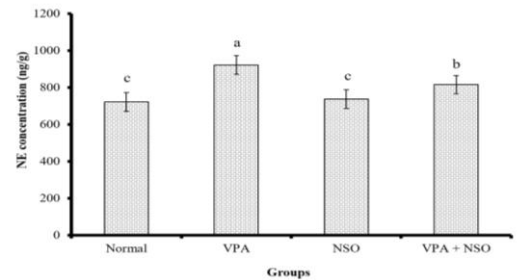


Fig. 3: The effect of NSO extract on homogenate NE concentration of VPA-administered albino rats.

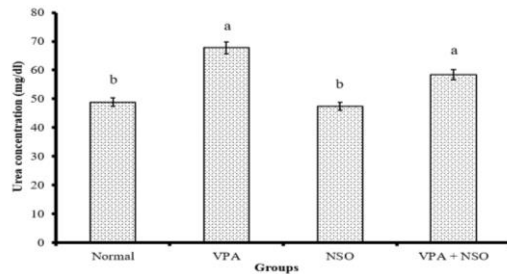


Fig. 7: The effect of NSO extract on serum urea concentration of VPA-administered albino rats.

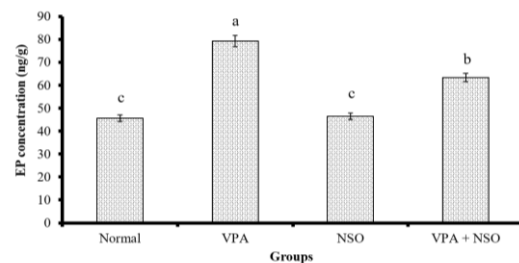


Fig. 4: The effect of NSO extract on homogenate EP concentration of VPA-administered albino rats.

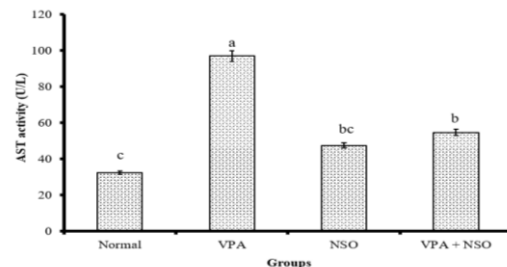


Fig. 8: The effect of NSO extract on serum AST activity of VPA-administered albino rats.

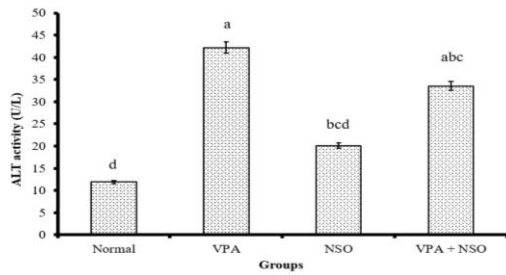


Fig. 9. The effect of NSO extract on serum ALT activity of VPA-administered albino rats.

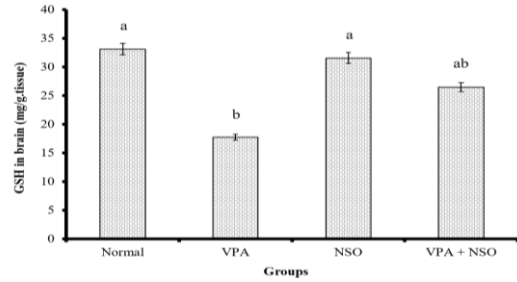


Fig. 14. The effect of NSO extract on brain GSH content of VPA-administered albino rats.

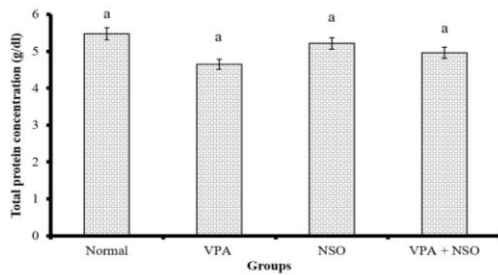


Fig. 10. The effect of NSO extract on serum total protein concentration of VPA-administered albino rats.

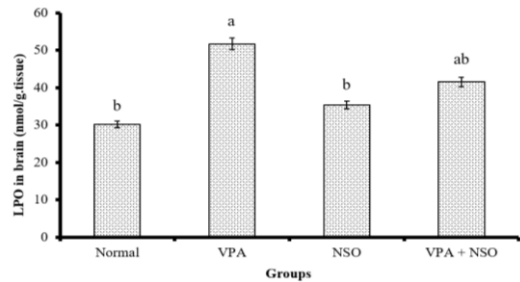


Fig. 15. The effect of NSO extract on brain LPO of VPA-administered albino rats.

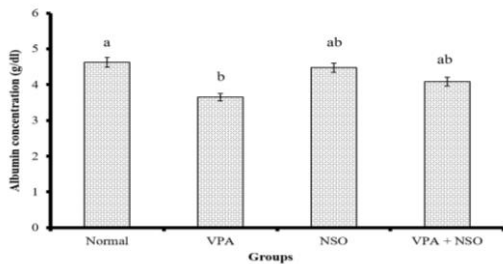


Fig. 11. The effect of NSO extract on serum albumin concentration of VPA-administered albino rats.

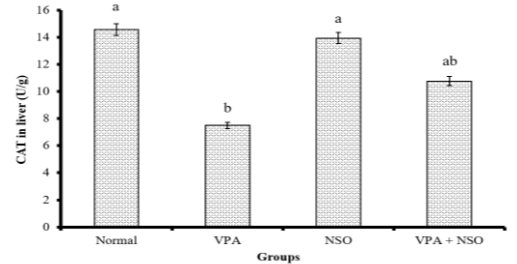


Fig. 16. The effect of NSO extract on liver CAT activity of VPA-administered albino rats.

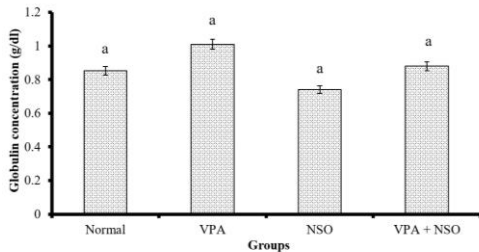


Fig. 12. The effect of NSO extract on serum globulin concentration of VPA-administered albino rats.

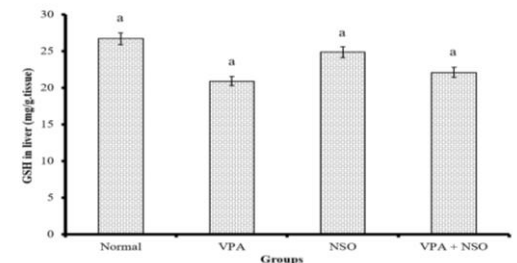


Fig. 17. The effect of NSO extract on liver GSH content of VPA-administered albino rats.

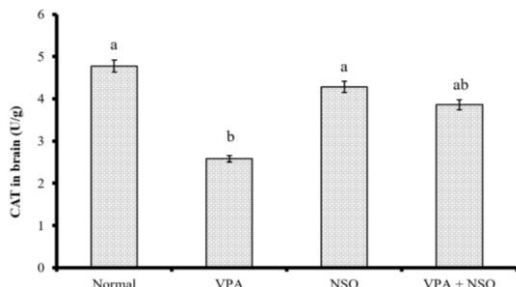


Fig. 13. The effect of NSO extract on brain CAT activity of VPA-administered albino rats.

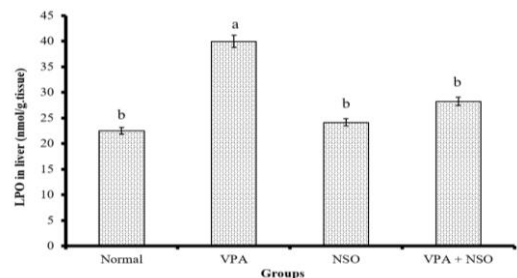


Fig. 18. The effect of NSO extract on liver LPO of VPA-administered albino rats.

Discussion

VPA, a branched short-chain fatty acid, is generally used as an antiepileptic drug and a mood stabilizer. VPA is a relatively safe drug, but its use in higher concentrations is associated with idiosyncratic neurotoxicity (Chaudhary and Parvez, 2012), hyperammonemia encephalopathy (VHE) (Larsen and Østergaard, 2014) and hepatotoxicity (Amrani *et al.*, 2013).

Our study was concluded the potential role of oxidative stress in VPA-induced neurotoxicity for the purpose of evaluating its participation in the brain damage mechanisms responsible for the neurological impairment and in VAP-induced hepatotoxicity by liver damaging. Interestingly, NS has shown protection against epilepsy and convulsions in few animal studies (de Almeida *et al.*, 2011). In this present study, we showed the effect of treatment with NSO extract on VPA-administered rats.

The enzyme family of cholinesterases contains the acetylcholinesterase (AChE), also known as true, specific, or type I cholinesterase, and the butyrylcholinesterase (BChE), also known as plasma, nonspecific, type II cholinesterase (Lampón *et al.*, 2012) pseudocholinesterase, or serum cholinesterase which hydrolyses both choline and aliphatic esters. BChE is an α -glycoprotein found in the central and peripheral nervous system, in most tissues, and in the liver (Santarpia *et al.*, 2013). Our study showed that, the administered VPA to albino rats produced a significant elevate in activity of serum BChE enzyme. (Lampón and Tutor, 2011) agree with our results, denoted that significant elevates of BChE activity in groups of patients with non-alcoholic fatty liver disease. It has been hypothesized that raised BChE activities could lead to a reduce of acetylcholine which has anti-inflammatory actions and therefore could trigger the onset of low-grade systemic inflammation (Das, 2007 and Rao *et al.*, 2007). Elevated BChE activities have been described in distinct pathophysiological diseases such as obesity, hyperlipidemia, hypertension, insulin resistance, metabolic syndrome, liver steatosis, diabetes mellitus or Alzheimer disease (AD) (Lampón *et al.*, 2012). BChE is associated mainly with glial cells, in addition to endothelial cells and neurons (Darvesh *et al.*, 2003). Modification in the levels of BChE in cases wherein there is a deficiency or absence of AChE supposes significance in clinical effects in which AChE insufficiency occurs such as Alzheimer's disease. Before an example, in Alzheimer's disease, AChE is lost up to 85% in specific brain regions, whereas BChE levels, especially the G1 form, rise with disease progression. In the Alzheimer's disease, elevating levels of BChE correlate significantly and positively with the improvement of hallmark cortical and neocortical amyloid rich neuritic plaques and neurofibrillary tangles. Although the accurate role of β -amyloid peptide, which collects in neuritic plaques, is not well understood, it is supposed that it is toxic to neurons (Vijaya *et al.*, 2015). Our study indicated that treatment of administered VPA rats with NSO extract, exhibited a significant raise in BChE enzyme with normal group. These results agree with Yassin (2005) concluded that, NS manifested pronounced recovery in the activity of serum BChE against serum of lead intoxicated rabbits. Ismail *et al.*, (2008) suggested that NSO improves memory in this model perhaps through mechanism(s) independent of the cholinesterase enzyme inhibition as its neuroprotective

influences against beta amyloid ($A\beta$) toxicity may play a possible role in preventing Alzheimer's disease (AD) advancement.

Neurotransmitters are signalling molecules in the nervous system. Their role as signalling molecules depends on receptors that are certain to each neurotransmitter in the synaptic cleft. These molecules are key factors in an extensive range of behaviours (Narvaes and de Almeida, 2014). Our data showed that, the high dose of VPA intake caused a very significant increase in the homogenate 5-HT, NE, EP and DA concentrations of rats-administered VPA as compared to normal rats. These results agree with Abdel-Rahman *et al.*, (2013). One of the mechanisms that have been greatly obtained for the action of VPA is the raise of the brain levels of the inhibitory neurotransmitter γ -amino butyric acid (GABA) by inhibiting GABA transaminase and thus preventing GABA metabolism. In addition to GABA, it has also been suggested that VPA may strive its anticonvulsant effect through the regulation of monoamine neurotransmitters (Johannessen, 2000 and Rosenberg, 2007). According to unanimous belief, increased levels of 5-HT, NE and DA in the brain strive an anticonvulsant activity (Abdel-Rahman *et al.*, 2013). The data about sodium valproate as established anticonvulsant drug is moderated by alteration in monoamine levels in rat brain areas as the improvement of monoaminergic transmission decreased seizure threshold (Abdel-Rahman *et al.*, 2013). This may be explained through valproate mechanism via activation of monoamine oxidase (MAO) the key enzyme that degrades a number of monoamine neurotransmitters (oxidizes 5-HT, NE, EP, and DA and is irreversibly limited by low concentrations of clorgyline as newly reproduced by Wu and Shih (2011). The potential mechanisms by which VAP controls the serotonin level may comprise distinct serotonin regulators in the serotonergic system. Preceding studies have detected that VPA is capable of affecting the activities of tryptophan hydroxylase, the enzyme responsible for serotonin synthesis, and definite subtypes of serotonin receptors such as 5-HT_{2A} and 5-HT_{1B} receptors. MAO uses serotonin as a substrate, and MAO dysfunctions associated with a serotonin imbalance have been involved in various mental disorders comprising depression, social anxiety, and autism (Wu and Shih, 2011). The present study revealed that, the treatment with NS alleviate the modifications in monoamines (5-HT, NE, EP and DA) treated rats, these results agree with Abdel-Rahman *et al.*, (2013) investigated that the neuroprotective role of NS extract on ciprofloxacin and pentylenetetrazole treated rats, these findings reflect the powerful antiepileptic activity of NS as suggested by Ezz *et al.*, (2011). Moreover, studies executed by Perveen *et al.*, (2008 and 2009) stated that the administration of NSO elevated the 5-HT, tryptophan levels and reduced levels of 5-HIAA in the rat brain suggesting a reduced 5-HT turnover supporting its anti-anxiety effect, these may demonstrate the raise in monoamines content in the present study. TQ, the essential component of NS seeds extended the onset of Pentylenetetrazole (PTZ)-induced seizures and decreased the duration of myoclonic seizures and hypothesized TQ anticonvulsant activity in the petit mal epilepsy through an elevated in GABAergic tone (Hosseinzadeh and Parvardeh, 2004).

Amino acids, products of protein breakdown, are commonly metabolized in the liver by transaminases and oxidative deaminases to cause NH_4^+ (Mittal *et al.*, 2009). NH_4^+ is then changed to urea (a by-product from protein breakdown) and about 90% of urea produced is excreted through the kidney (Walmsley *et al.*, 2010). The present results illustrated a significant raise in NH_4^+ and urea concentrations in serum of administration of VPA as compared to normal rats. These results run parallel to these of another investigators (Lee *et al.*, 2015). The raised levels of NH_4^+ and urea in the plasma of sodium valproate-administered could be due to valproate-induced VHE (Segura-Bruna *et al.*, 2006). Hyperammonemia (HA) induced by VPA have not yet been fully cleared. Studies proposed a hepatic and renal role: amino acids are degenerated in the liver to produce NH_4^+ (Cash *et al.*, 2010). Any alteration in this process may compromise the urea cycle and induce HA. In the kidney, VPA influences the renal uptake of glutamine elevating the NH_4^+ production (Carr and Sherwsbury, 2007). Brusilow (2002), in a narrative review on HA, enhanced how NH_4^+ is considered to purpose encephalopathy. Within the central nervous system (CNS), raised NH_4^+ leads to higher production and gathering of glutamine within astrocytes. This elevated intracellular glutamine leads to cerebral edema and astrocyte dysfunction. The mechanisms by which the brain is thought to recompense for astrocyte swelling in chronic HA comprise minimized osmolarity and thus edema by down-regulation of *myo*-inositol, elevated brain tissue compliance, and mild to temperate brain atrophy (Brusilow, 2002). The present results showed a non-significant in treated with NSO extract in serum of NH_4^+ concentration but ameliorations noticed and a significant increase in treated with NSO extract in serum of urea concentration. This is in agreement with Mohammed *et al.*, (2014) concluded that, treatment with NSO significantly reduced the influences of cadmium-induced damage in the kidney tissues, and it was confirmed by the inhibited level of blood urea in rats. Also, agreement with Khan *et al.*, (2013) reported that, the NS aqueous solution and NSO extract administration reveals hepatoprotective and nephroprotective effects on acetaminophen induced liver and renal injuries to chicks. NSO extract treated group demonstrated greater resistance against hepatic and renal injuries which is noticeable by almost normal concentration of serum urea. It is deduced that NS has positive influences on urea concentration due to NS seeds (and some of its active components, e.g. volatile oil and TQ) have been reported to supply protection against nephrotoxicity and hepatotoxicity induced by some toxins or anti-cancer drugs. NSO extract may be impacted on serum of NH_4^+ concentration by reduced level of treated group.

Our data showed that, administration of VPA induced hepatic dysfunction detected by a significant raise in serum of AST and ALT activities, the treatment with NSO extracts demonstrated a manifest improvement in activity of VPA-administered treated groups as compared to their corresponding normal ones. These results agree with (Eman *et al.*, 2015). Such elevate in AST and ALT may be due to the damaged hepatocytes and bile duct proliferation noticed in this work (Okdah and Ibrahim, 2014). Ibrahim (2012), reported that VPA induces toxic influences on liver tissue of mice which exhibited vacuolar degenerative changes, hypertrophied nucleus with fragmented chromatin, inflammatory cells collects

and congested vasculature. Treatment of VPA with NSO extracts include of TQ (A major component t of volatile oil of *Nigella* species) is an antioxidant and keeps liver against VPA induced toxic damage. TQ significantly declined the AST and ALT activities (Raza *et al.*, 2006).

The present study indicated that total protein and globulin were showed non-significantly, on other hand albumin was depleted significantly in rats-administered VPA. Ibrahim (2012) reported that significantly reduction of protein content and deduced that the administration of VPA produces evaluation of hepatotoxicity of VPA in albino mice by Histological and histoistochemical studies. Abdel-Dayem *et al.*, (2014) and Eman *et al.*, (2015) concluded that especially decreased in serum albumin level in rats by taken VPA, and also Rugino *et al.*, (2003) showed that, the serum albumin level in five patients was significantly reduced when VPA was administered and reported that VPA was associated with the progression of hypoalbuminemia. Shaalan *et al.*, (2015) noticed that, serum total protein, albumin and globulin were declined after chronic administration of VPA. VPA is widely ($\geq 90\%$) bound to plasma proteins, chiefly albumin, correspondingly to endogenous free fatty acids (FFA). The extent of binding lowers with raising drug concentration, resulting in an elevate of the free fraction of the drug (Silva *et al.*, 2008). The present study explained that rats kept on oral intubation of extract of NS cause non-significantly in total protein, albumin and globulin. Hagag *et al.*, (2013) reported that There was no significant difference in the total serum protein, albumin, globulin levels in protective impact of NSO against methotrexate induced hepatotoxicity in children with acute lymphoblastic leukemia. This could be demonstrated this by the efficiency of the liver to raise protein, albumin biosynthesis during diseases associated with protein loss, or in presence of liver cell damage or injury induced by cytotoxic drugs, until the parenchymal damage or loss is serious. Also, Mohamed *et al.*, (2010) showed that no significant variation in total proteins, and serum albumin between NS seeds against dimethylaminoazobenzene (DAB) induced liver carcinogenesis. On the other hand, the results of (Al-Kadhi, 2014) was against to our results that it showed that there was a significant elevated in serum globulin levels administrated of NSO.

Oxidative stress is realized as an imbalance between higher cellular levels of reactive oxygen species (ROS, e.g., such as superoxide, radical, $\text{O}_2^{\cdot -}$; hydrogen peroxide, H_2O_2 ; hydroxyl radical, HO^{\cdot}) and the cellular antioxidant defense. In order to scavenge reactive oxygen species, various defense systems remain in the brain, such as enzymatic (CAT), non-enzymatic (GSH) antioxidants. (Ihan *et al.*, 2005). Generation of free radicals may be, at least partially, the foundation of abundant neurological and neurodegenerative disorders such as ischemia-reperfusion, seizure, Parkinson's and Alzheimer's disease and antioxidant therapy have been well registered to protect against CNS injuries (Hosseinzadeh *et al.*, 2007).

In this study found that, VPA significantly lower the activity of CAT activity. These results are agreement with Chaudhary and Parvez, (2012), found a significant decreased of CAT activity in the cerebellum and cerebral cortex of rats. Valporate-induced toxicity implicating hepatotoxicity by Vidya and Subramanian (2006),

explained that VPA-administered was found to diminished levels of enzymatic CAT antioxidants in rats. The reduced CAT activity induced by VPA may be due to the flux of superoxide radicals. (Freitas *et al.*, 2004). In the present work showed that NSO treatment was a non-significant in both brain and liver CAT activity as compared to the normal rats but an amelioration existed. In results of (Kanter *et al.*, 2006) pronounced that prevented reduction of CAT enzyme activity following experimental spinal cord injury in rats, and also results of (Sultan *et al.*, 2014) reported that, Use of NS fixed and essential oils resulted in noticeable elevate in the activity of CAT liver enzyme against streptozotocin (STZ) induced hepatotoxicity in diabetic rats.

In this investigation showed that, VPA is a significantly decreased the content of GSH in brain and but a non-significantly changed in liver. this study agrees with Chaudhary and Parvez (2012), found a significant decreased the total content of GSH in the cerebellum and cerebral cortex of rats, but disagreement with Amrani *et al.*, (2013), reported that depletion of the antioxidant GSH has been suggested as one of the mechanisms leading to VPA-associated hepatotoxicity. Treatment with NSO was resulted a non-significant in the brain and hepatic GSH levels when compared to normal ones. This offered that NS acts by raising activity of antioxidant or lowering oxidation and thus may provide to elevated availability of GSH to act against raised oxidative stress and showing reduction in free radical induced oxidation (Sandhu and Rana, 2013). TQ, is already supposed to be an antioxidant. Although fractions rich in TQ were established to be most efficient in terms of antioxidant capacity, however, previous research elucidates that the protective effects of NS may not only be due to TQ, but perhaps also due to other antioxidants (Azzubaidi *et al.*, 2012).

In the present study, we first noticed that VPA significantly increased the level of LPO dose dependently in brain and liver tissues. A study by (Chaudhary and Parvez, 2012) led to a significant induction of LPO content in rat brain. Oxidative stress in liver was stimulated by VPA significant increase in LPO which could penetrate the biologic membranes, were perceived. LPO has been hypothesized to be a major mechanism of cell damage by free radicals. The acquired data revealed that the significant elevate in the level of LPO may be due to its poor antioxidant defense or the inactivation of antioxidant enzymes due to oxidative stress. A study by Leipnitz *et al.* (2010) also found that branched chain fatty acids (BCFAs) like phytanic acid caused a significant induction of both LPO content in cerebellum as well as in cerebral cortex of rat brain. This result is consistent with preceding report (Raza *et al.*, 2000). Raza *et al.* (2000) showed that malondialdehyde raised in liver of mice fed with sodium valproate for 21 days. In this investigation, It has been shown that NSO inhibits a non-enzymatic LPO. Burits and Bucar (2000) showed that NSO as well as its compounds, essentially TQ, have considerable antioxidant and free radical scavenger properties but no pro-oxidant effect. The antioxidant action of NSO may demonstrate the protective effect of these agents against different neurotoxic and hepatotoxic models *in vivo* and *in vitro* (Hosseinzadeh *et al.*, 2007). Hosseinzadeh *et al.*, (2007) showed that NSO and its active constituent TQ, have inhibitory effects against LPO process during cerebral ischemia-reperfusion injury in rat hippocampus.

Kanter *et al.*, (2005) has also reported that NSO lowered the LPO and raised the antioxidant defense system activity in the CCl₄-treated rats.

Conclusion

In conclusion, VPA administration alters biochemical parameters, increases the formation of oxygen free radicals, reduces antioxidants (CAT, GSH) and increases LPO which leads to organ damage. Treatment with NSO extract (after VPA administration) reduced the changes in oxidative stress markers and succeeded in amelioration and improvement of the altered biochemical and oxidative-antioxidant parameters to nearly those of the control group. A possible protective effect of treatments on VPA induced neurotoxicity and hepatotoxicity may be explained on the basis of oxidant-antioxidant system management. Thus, NSO extract may act as a useful therapeutic agent in preventing toxicity which may occur after VPA treatment. However, further clinical studies to prove this effect is also needed for its applicability in humans for treatment of toxicity induced by VPA.

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