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SOME BIOLOGICAL AND GENETIC STUDIES ON RATS TREATED WITH ACRYLAMIDE WITH OR WITHOUT *NIGELLLA SATIVA* WITH SPECIAL REFERENCE TO THEIR EFFECTS ON GLUTATHIONE-S-TRANSFERASE AND REDUCED GLUTATHIONE

S. HEDAYA*, A. MANDOUR*, KH. ASHRY** and SH. HEMEDA***
Department of Physiology and Biochemistry*
Department of Veterinary Forensic Medicine and Toxicology**
Department of Animal Husbandry***
Fac. Vet.Med., Alex. Univ.

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SUMMARY

The present study was conducted to explore the effects of acrylamide (ACR) and *Nigella sativa* (N.S) (which was used as a natural protection) on biochemical, hematological and genetic profiles of Albino rats. The obtained data showed that the lowest values of serum enzymatic activities [Asparatate and Alanine amino transferase (AST, ALT); Gamma glutamyl transferase, (GGT) and Lactate dehydrogenase (LDH) were noticed in sera of rats treated with (ACR) followed by those given (N.S) with (ACR). Furthermore, the glutathione-S-transferase and reduced glutathione (GSH) values were also significantly reduced in liver and kidneys of both groups treated with (ACR) after one and two weeks. Insignificant increase in activities of all studied enzymes as well as reduced glutathione (GSH) was recorded in rats treated by Nigella sativa only. On the other hand, the lowest values of packed cell volume (PCV), hoemoglobin (Hb), RBCs, WBCs, acidophils and lymphocytes were observed in (ACR) treated group allover the experimental period, these results nearly reversed in rats treated with Nigella sativa only. A slight improvement in biochemical and hematological criteria was observed in rats given (ACR) plus (N.S). The obtained results revealed a significant decrease in the rate of mitosis in (ACR) treated group during the experimental period. The chromosomal structure and number is significantly altered in both groups treated with Acrylamide. Finally, the microscopic and ultra structural changes were observed only in the 14^{th} day of experiment especially in rats treated with (ACR) and (ACR) plus (N.S). Furherly encountered lesions were more severe in (ACR) treated group than those given (ACR) plus (NS).

INTRODUCTION

Acrylamide is used in the production of polymers and grouting and in the stabilization of soil used to construct dams, tunnels, and foundations (Davis et al., 1976). It is also used as a coagulant in the treatment of potable water as a flocculant in the treatment of waste water and in electrophoresis and chromatography.

It was found that, acrylamide produces neurotoxic, genotoxic, reproductive, carcinogenic and immunosuppressive effects in laboratory animals (Butterworth et al., 1992; Matsuoka et al., 1993; Abou Donia et al., 1993; Tsuda et al., 1993 and Zaidi et al., 1994). Some enzymes were found to be inhibited by (ACR) as glyceraldehyde-3 phosphate dehydrogenase (Orstan and Gafni, 1990); brain creatine kinase (Kohriyama et al., 1994), several enzymes of glycolysis both in vitro and in vivo (Vyas et al., 1985). Furtherly, Burek et al., (1980) found that the body weight, serum cholinesterase, packed cell volume, red blood cells and hemoglobin were decreased in ACR treated animals. Also, the reduced glutathione is reduced after ACR administration (Beiswanger et al., 1993). Tsuda et al., (1993) observed different type of chromosomal aberrations induced by ACR. Recently, Helal et al., (1998) concluded that Nigella sativa oil has an immunostimulant effect. Also, Rady et al., (1997) stated that the NS oil inhibited the non-enzymatic peroxidation in chicken RBCs, that suggests the antioxidant activity of this oil. So, this study was planned to throw light on the adverse effects of acrylamide on biochemical, hematological and genetic profile of blood and tissues of albino rats with the possible using of Nigella sativa as an natural antitoxic agent.

MATERIAL AND METHODS

Chemicals: Acrylamide (2-Propenamide > 99.9% HPLC) was obtained from Sigma Chemical Co. It was given in drinking water at a dose rate of 0.05% (such dose which produce neurotoxic lesion after 10-12 days, Abou-Donia et al., 1993). Nigella Sativa seeds, was crushed and its methanolic extract was prepared according to Salomi et al., (1991) and injected S/C at a dose of 0.4ml/Kg B.wt. twice weekly for two weeks

Animals: Eighty mature male albino rats (200-240g) were housed in plastic cages (5 animals/cage) . Animals were provided with feed pellets and tap water adlibitum. Rats were divided into four equal groups as follow:-

GroupI: Injected S/C with 0.4ml saline/Kg B.wt. and serve as control group.

GroupII: Was administered with 0.4ml/Kg B.wt (S/C) of Nigella sativa extract (freshly prepared) twice/week for two weeks.

Group III: Was given acrylamide in drinking water daily at a dose of 0.05% for two weeks,

and injected also with saline as group I .

Group IV: This group was given ACR as group III and NS extract as group II for two weeks.

Blood Sampling:-

Two ml of blood were taken from the orbital sinus of each rat after one and two weeks (10 rats from each group weekly). $^1/_2$ ml of them was transferred in a clean dry bottle containing EDTA (1mg/5ml blood) as anticoagulant for determination of hemoglobin, packed cell volume, RBCs count, leucocytic count and differential leucocytic count, (Schalm et al., 1975). The remainder blood was left to clot, then centrifuged at 1500 r.p.m for 10 minutes and the obtained non hemolyzed clear sera were used for quantitative determination of AST and ALT (Reitman and Frankel, 1957); GGT (Szasz, 1974) and LDH as mentioned in Wootton (1982) . After that rats were killed by cervical dislocation and the organs (Liver and Kidneys) were perfused with ice-cold 0.05 M tris-HCI buffer pH 7.4 containing 0.25 M sucrose and the organs were excised and placed in the same ice-cold buffer. The organs were blotted dried, weighted, and then homogenized in the ice cold buffer with twelve stroks in a tight-fitting Potter Elvehagen homogenizer. Glutathione-S-transferase activity was determined sepectrophotometrically at room temperature after Habig et al., (1974). Also the reduced GSH was determined in the homogenates after (Sedlak and Lindsay, 1968) using Ellman's reagent.

For cytogenic study, the preparation of metaphase chromosomes, splash technique was employed as described by Macgregor and Varley (1983). For calculation of mitotic index, about 2000 cells of each group were examined to detect the number of divided and non divided cells where mitotic index (M.I) is equal to:

M.I = The number of divided cells/Total number of divided and non divided cells.

Moreover, about 500 well spread metaphase figure (100 of each animal) were examined for detection of different type of chromosomal aberrations.

Statistical analysis: of the obtained data were calculated and analyzed by using F-test (Snedecor and Cochran 1971) . Also the Chi-square test of the Epi-Info computer package (Epi-Info, 1994) was used to determine the significant differences between the control and treated groups.

For histopathological studies, very small specimens from lumber region of spinal cord and sciatic nerve (I mm) were fixed in 5% cold glutarylaldehyde dehydrated in ethanol and embedded in epon. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined in transmission electron microscope to determine the ultra structural changes. In the same time, another specimens from brain, spinal cord and sciatic nerve were fixed in 10% neutral buffered formaline, dehydrated in alcohol, cleared in xylene and embeded in paraffein wax 0.5

microns thick sections were stained by hematoxylin and eosin (H & E) and examined in light microscope to evaluate the histopathological lesions (Lillie, 1954).

RESULTS

The signs of neurotoxicity were clear at the end of the 2 weeks of treatment with ACR. The rats exhibited splay of the hind limb, change in gait and finally hind leg paralysis. These signs were less pronounced in rats treated with ACR plus NS extract. The obtained data were listed in five tables and showed in seven figures. Table. (1,2) showed that, the activities of all studied enzymes in serum and tissues were significantly reduced in ACR and ACR+N.S treated groups.Although the activities of these enzymes (ALT, AST, GGT, LDH and Glutathione-S-transferase) were increased in the last group (ACR+N.S) than those in ACR treated group, their activities still significantly ($P<0.05$) lower than those in control group. Reduced glutathione follows the same trend of the enzymes (Table 1) after one and two weeks.

Insignificant increase in all enzymatic activities as well as glutathione were noticed in rats injected only with Nigella Sativa extract (Table 1,2).

Concerning hematological picture, Table (3) demonstrated that, all parameters of blood picture were decreased significantly ($P = <0.05$) in rats given ACR except neutrophils which was significantly increased while, monocytes and basophils showed a non significant variation with control group. These obtained data for ACR treated group was completely reversed in N.S. injected rats. A noticeable improvement was observed for most blood parameters towards those in control group in rats treated with combination of ACR and NS. Table (4) and Fig. 1 (a,b) showed that, there were a significant decrease in the rate of mitosis in ACR treated groups (III and IV) after one and two weeks. On the other hand, Nigella sativa has a non significant effect on the rate of mitosis. Table (5) and Fig. (2) represented the different type of aberrations which appeared in both groups

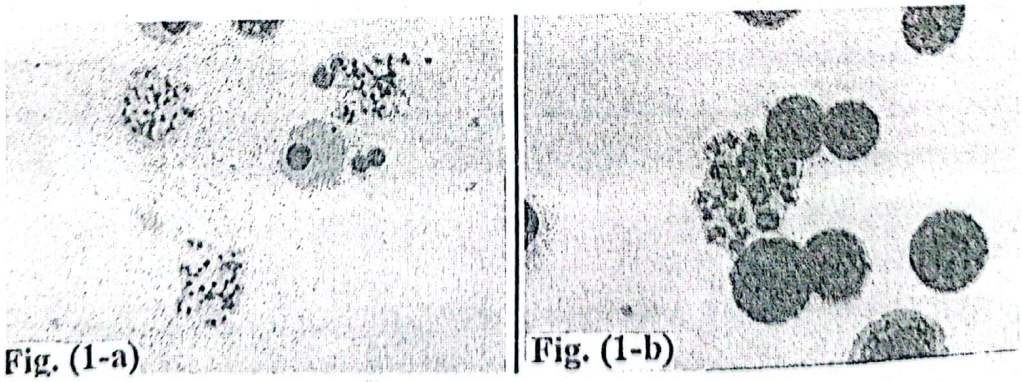

Fig. 1: Showing (a) normal mitotic acitivity and (b) mitotic inhibition of bone marrow cells of treated rats.

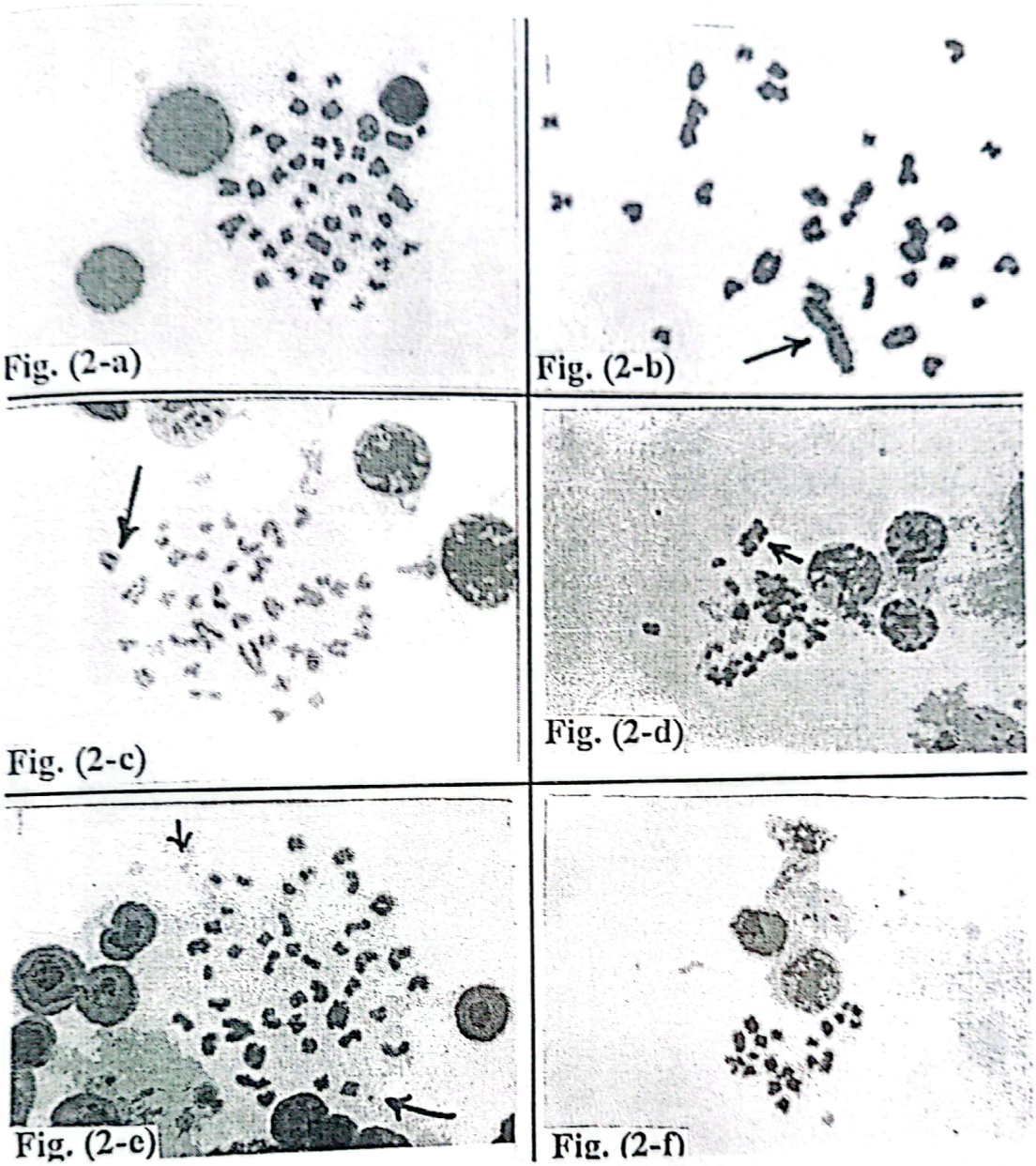

Fig. 2: Showing (a) normal metaphase chromosomes, and aberrant chromosomes; (b) chromosomal stickness (Tandam Fushion), (c) deletion, (d) breaks (e) fragments, and (f) Heteroploid cells in treated rats.

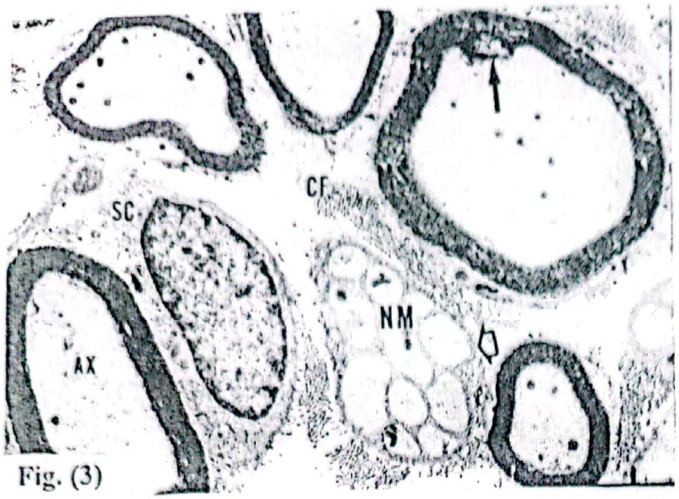

Fig. (3)

Fig. 3: Electron micrograph of Sciatic nerve of ACR + NS treated group: Slight separation of the myelin lamellae (↑) of myelinated axon, myelin sheath (MS), axoplasm (AX), schwann cell (SC), unmylinated axons (NM) surrounded by cytoplasm of schwann cell ( ). Notice large amount of collagen fibers (CF) (X 10.000).

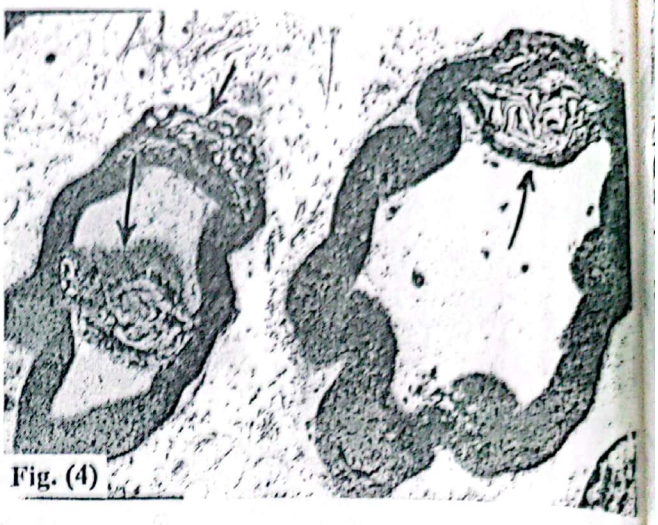
Fig. (4)

Fig. 4: Electron micrograph of sciatic nerve of ACR treated rats: Severe disintegration of the myelin sheath lamellae of myelinated axons (arrows) with loss of axolemmal mitochondria and neurofilament of the axon to the right X 8000.

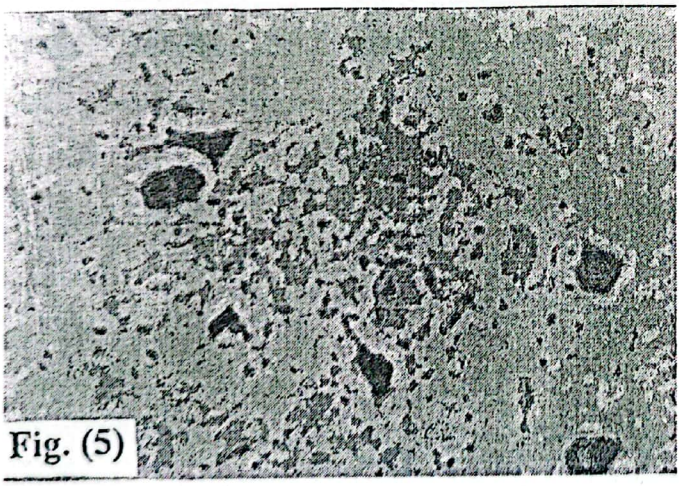
Fig. (5)

Fig. 5: Brain of ACR treated group: Neural degeneration (Pyknosis and tigrolysis) in the cerebral cortex (H & E X 250).

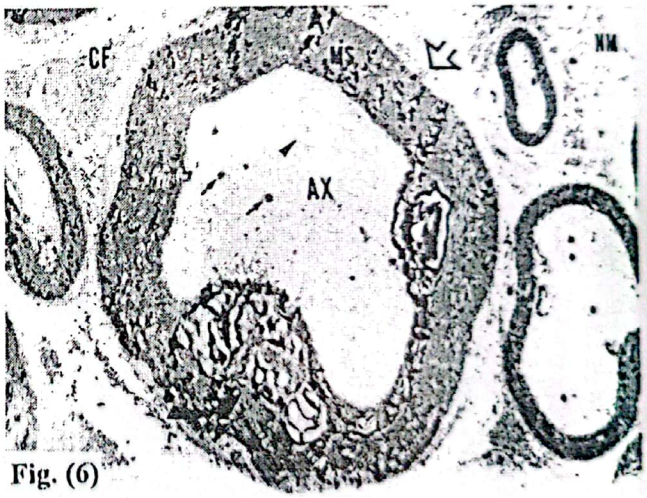

Fig. (6)

Fig. 6: Electron micrograph of spinal cord of ACR + NS treated group: Focal separation of Lamallae (↗) of myelin sheath (MS), axoplasm (AX) containing neurofilaments (Δ) and fine neurotubules (↑). The myelin sheath was surrounded by cytoplasm of schwann cells (↑), unmyelinated nerve fibers (NM). Notice large amount of collagen fibers (CF X 8000).

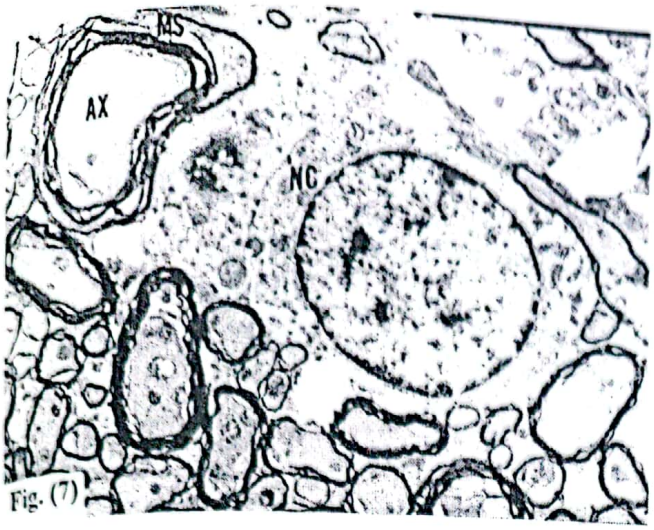

Fig. (7)

Fig. 7: Electron micrograph of spinal cord of ACR treated group: Severe disintegration of the myelin sheath (MS) axoplasm (AX) and nerve cell (NS X 8000.

treated with ACR (III and IV). Nigella sativa had a non significant effects on induction of chromosomal aberrations as compared with those in control group. Regarding, the histopathological examinations, the microscopic and ultrastructural changes was clearly observed on the 14 th day of experiment, lesions were observed only in rats given ACR and ACR + NS. The lesions were more severe in rats treated with ACR only than that of rats given ACR +NS. The lesions were described in figures (3-7).

Table (1): Glutathione-S-transferase activity and reduced glutathione values in liver and kidney of rats treated with acrylamide with or without Nigella sativa extract for one and two weeks.

Period	After one week				After two weeks			
Parameter	Liver		Kidney		Liver		Kidney	
	GST	GSH	GST	GSH	GST	GSH	GST	GSH
G I*	2980.81±296.46^{a}	169.32±6.78^{a}	762.4±43.09^{a}	68.74±5.60^{a}	3191.22±286.61^{a}	186.6±6.58^{a}	789.00±61.33^{a}	73.71±7.98^{a}
G II	3254.18±460.14^{a}	180.36±8.22^{a}	800.22±59.48^{a}	77.59±5.09^{a}	3396.81±270.68^{a}	208.24±16.21^{a}	853.81±90.36^{a}	77.95±5.62^{a}
GIII	2161.18±240.18^{b}	86.20±5.34^{b}	518.47±38.6^{b}	45.64±4.26^{b}	2048.59±190.20^{b}	116.09±11.97^{b}	400.47±59.40^{b}	46.51±6.58^{b}
G IV	2539.35±300.34^{b}	105.29±8.60^{b}	602.07±41.7b	51.88±5.90^{b}	2156.20±210.11^{b}	130.61±15.99^{b}	430.81±80.45^{b}	50.00±4.94^{b}

- Data are expresed as Means ± S.D.
- Values with different letter are significantly different at (P<0.05).
- Glutathione-S-transferase (GST) & reduced Glutathione (GSH) values were expressed as µmoles substrate/min/mg protein and µmoles/g wet ts. respectively.

Table (2): Serum enzymatic activities in rats treated with acrylamide with or without Ni gella sativa extract after one and two weeks.

Parameter	After one week				After two weeks			
	AST *U/L*	ALT *U/L*	GGT *U/L*	LDH *U/L*	AST *U/L*	ALT *U/L*	GT *U/L*	LDH *U/L*
G I*	76.00±4.69^{a}	66.8±5.15^{a}	5.62±0.24^{a}	652±53.10^{a}	68.45±4.15^{a}	49.60±7.66^{a}	5.98±0.98^{a}	724.8±80.11^{a}
G II	80.6±7.39^{a}	69.7±6.53^{a}	6.08±0.51^{a}	700±56.30^{a}	74.32±5.77^{a}	56.80±7.30^{a}	6.40±0.89^{a}	760.6±71.09^{a}
GIII	39.20±5.42^{b}	31.86±3.87^{b}	4.10±0.38^{b}	382.44±40.22^{bc}	31.80±6.79^{b}	19.00±3.10^{b}	3.24±0.49^{c}	317.66±40.9^{b}
G IV	47.60±5.12^{b}	40.96±4.36^{b}	4.31±0.99^{b}	415.6±59.03^{b}	46.40±5.38^{b}	30.81±4.81^{b}	4.78±0.61^{b}	409.30±51.46^{b}

* Animal group as described in method section.
- Data are expresed as Means ± S.D.
- Values with different letter are significantly different at (P<0.05).
- AST: aspartate amino transferase, ALT = alanine amino transferase , GGT = gama glutamyl transferase .LDH = Letlate dehydrogenase lactate dehydrogenase.

Table (3): Blood picture of rats treated with acrylamide with or without Nigella sativa extract for one and two weeks.

	After one week									After two week								
	PCV %	Hb g/dl	RBCs	WBCs	N%	E%	B%	Ly%	M%	PCV %	Hb g/dl	RBCs	WBCs	N%	E%	B%	Ly%	M%
G I*	39.4 ±2.07^{a}	13.4 ±1.16^{ab}	6.00 ±0.4^{a}	5.80 ±0.66^{a}	25.0 ±0.82^{b}	3.0 ±0.5^{a}	1.2 ±0.01^{a}	66.4 ±4.10^{a}	4.4 ±0.6^{a}	39.60 ±3.05^{a}	13.9 ±2.06^{a}	5.90 ±0.8^{a}	6.20 ±0.81^{a}	25.4 ±2.0^{bc}	3.1 ±0.42^{a}	1.2 ±0.33^{a}	66.30 ±4.5^{a}	4.0 ±0.41^{a}
G II	42.8 ±3.20a	14.5 ±1.50^{a}	6.20 ±0.99^{a}	6.20 ±0.81^{a}	21.1 ±2.00^{b}	3.4 ±0.4^{a}	1.3 ±0.3^{a}	71.2 ±6.5^{a}	3.0 ±0.8^{b}	42.90 ±4.00^{a}	14.8 ±3.11^{a}	6.50 ±1.36^{a}	6.50 ±0.64^{a}	22.0 ±1.00^{c}	3.0 ±0.62^{a}	1.0 ±0.0^{a}	70.0 ±5.0^{a}	4.0 ±1.66^{a}
GIII	30.2 ±1.9^{b}	10.0 ±1.38^{b}	5.10 ±0.61^{b}	4.20 ±0.47^{b}	38.0 ±3.00^{a}	1.6 ±0.65^{b}	1.2 ±0.18^{a}	55.0 ±6.11^{b}	4.2 ±1.09^{a}	30.40 ±2.22^{b}	9.3 ±1.77^{b}	4.95 ±1.8^{b}	4.00 ±0.34^{b}	43.4 ±2.4^{a}	1.4 ±0.49^{b}	1.0 ±0.01^{a}	50.0 ±5.17^{b}	4.2 ±0.99^{a}
G IV	35.2 ±3.06ab	12.8 ±1.6^{ab}	5.50 ±0.36^{ab}	5.50 ±0.48^{a}	32.0 ±4.00^{a}	2.6 ±0.27^{a}	1.0 ±0.01^{a}	60.0 ±6.11^{ab}	4.4 ±0.77^{a}	36.06 ±3.5^{ab}	12.2 ±2.00^{a}	5.30 ±0.66^{ab}	5.70 ±0.98^{a}	31.4 ±1.9^{b}	2.4 ±0.21^{a}	1.0 ±0.11^{a}	61.4 ±6.7^{ab}	3.8 ±1.22^{a}

* Animal groups as described in method section.
- Data expressed as Means ± S.D.
- Values with different letter are significantly different at (P<0.05).
- N : Neutrophil - E : Eosinophils.
- Ly : Lymphocytes. - B: Basophils.
- M : Monocytes.
- RBCs and WBCs values per 10^{6} /mm^{3} and 10^{3} /mm^{3} respectively.

Table (4): The mitotic index of bone marrow cells of rats treated with acrylamide with or without Nigella sativa extract for one and two weeks.

Time	Animal group			
	GI	GII	GIII	GIV
After one week:-				
Number of examined cells	2000	2000	2000	2000
Number of divided cells	120	115	96	82
Number of non-divided cells	1880	1885	1916	1918
Chi square value	-	0.07	4.23*	7.14*
After two week:-				
Number of examined cells	2000	2000	2000	2000
Number of divided cells	132	108	75	76
Number of non-divided cells	1868	1892	1925	1924
Chi square value	-	0.56	10.46**	9.92**

- Animal groups as described in methods section.
* Significant at (P<0.05).
**Significant at (P<0.01).

Table (5): Chromosomal aberrations in rats treated with acrylamide with or without Nigella sativa extract for one and two weeks.

Period after treatment	Animal groups						
	GI	GII		GIII		GIV	
One week:-							
Normal cells	484	475		382		366	
Aberrant cells	16	25	Chi square	118	Chi square	134	Chi square
Tandam fusion	3	5	(1.08)	35	(101.18)**	39	(107.36)**
Chromosomal deletion	-	3	(0.02)	21	(26.29)**	19	(30.45)**
Chromosomal breaks	5	4	(1.34)	12	(19.46)**	22	(17.38)*
Chromosomal fragments	3	7	(0.00)	16	(3.65)	18	(4.56)*
Heteroploid cells	5	6	(0.91)	34	(7.37)*	36	(9.53)**
			(0.001)		(20.92)**		(22.89)**
Two week:-							
Normal cells	478	470		335		323	
Aberrant cells	22	30	(1.78)	165	(161.56)**	177	(164.36)**
Tandam fusion	4	7	(0.41)	45	(36.79)**	50	(42.16)**
Chromosomal deletion	1	6	(0.001)	36	(35.30)**	28	(26.74)**
Chromosomal breaks	5	4	(0.09)	16	(4.38)*	31	(18.01)**
Chromosomal fregments	5	7	(0.91)	31	(22.20)**	36	(27.32)**
Heteroploid cells	7	6	(0.001)	37	(23.88)**	32	(18.47)**

- Animal groups as described in method section.
* Significant at (P<0.05).
**Significant at (P<0.01).

DISCUSSION

The glutathion-S-transferase (GST) [E.C.2.5.1-18] constitutes a complex family that collectively metabolizes chemotheraputic drugs, carcinogens, environmental pollutants and xenthobiotic, (Bekett and Hayes, 1993). Furtherly, GST exhibits peroxidase-activity towards hydroperoxide and serves to combat oxidative stress. The aminotransferases are mainly distributed within the periportal hepatocytes as well GGT and as such these enzymes provide satisfactory markers to detect damage to this zone of the liver but they are poor at detecting the liver damage that may occur in centrilobular hepatocytes (Rappaport, 1980). Theoretically, plasma levels of GST should provide a sensitive and reliable indicator of damage of any part of the liver (Hiley et al., 1988). In the present study, the Acrylamide supplemented group possess the lowest values of ALT,AST,GGT and LDH after one and two weeks, table (2). Moreover, the hepatic and kidney GST and GSH are greatly reduced in the same group, table (1). It is beyond the shade of any doubt that ACR inhibited several enzymes

as creatine kinase in rat brain homogenates, (Kohriyama et al., 1994); Na-K ATPase in sciatic and tibial nerves (Lehning et al., 1994) and glyceraldhyde-3 phosphate dehydrogenase (Orstan and Gafni, 1990) who also concluded that, ACR covalently reacts with the active site, cysteine residues of the enzyme and subsequently induces a conformational changes in the enzyme. Furthermore ACR was found to inhibit many enzymes of glycolysis both in vitro and in vivo in muscles and liver (Vyas et al., 1985). Serum cholinesterase also was decreased after ACR treatment, beside these adverse effects on the enzymatic activities, ACR decreased significantly ($P<0.05$) the values of reduced glutathione. Beiswanger et al., (1993) reported that the conjugation with glutathione is a mechanism of detoxification of ACR. The inhibitory effect of ACR for the enzymatic activities may be the cause of significant reduction in their values in sera of ACR treated animals (Table 1,2). Some authors concluded that GST is inducible by some drugs and chemicals that offer protection against toxic compounds (Benson and Barretto, 1985). GST activity was augmented in mice fed dried powder of brussel sprouts, caffee, beans or tea leaves. So we try to use Nigella sativa as a natural protection against the adverse effects produced by ACR. Table (1,2) showed that the injection of NS alone resulted in insignificant increase in the activities of the enzymes under study as well GSH. Recently, Korshom (1998) found that the GST activity was increased in liver of rats treated with Nigella sativa extract, while it is not increased in kidney. Previous study on use of Nigella Sativa seed as antioxidant was done by Rady et al., (1997) who found that, the feeding of Nigella sativa seeds significantly increase the activity of GST in chicken erythrocytes. Although, the values of all enzymes and GSH were increased in rats treated with N.S plus ACR, these values still significantly lower than that of control group (table 1,2). These results gave an evidence that NS extract at dose (0.4ml/Kg B.Wt) fails to correct the deleterious effects of ACR.

Concerning the hematological picture, table (3) showed that, the administration of ACR to male rats for one and two weeks, decreased the values of PCV, Hb and RBCs count which came in close agreement with the resullts obtained by Burek et al., (1980). Acrylamide causes a cytogenetic effects in bone marrow, (Cihak and Vontorkova, 1988) lesions in liver (Peterson and Lech, 1987) and testicular damage and atrophy (Sakamoto and Hashimoto, 1986), these adverse effects of ACR may explain the reduction of PCV, Hb and RBCs count. Also, data listed in table (3) revealed that ACR decreased the WBCs, lymphocytes and acidophil percentage, while neutrophile was increased. Zaidi et al., (1994) observed a significant decrease in the weight of spleen, thymus, bone marrow and circulating blood lymphocyte in rats given ACR. This effect may be attributed to the antiallergic effect (Ishii et al., 1994)

and its effects on the bone marrow. Nigella sativa increased the values of PCV, Hb, RBCs count and lymphocyte percentage, while decrease neutrophil percentage (Table,3), a result which agree with that obtained by Satish et al., (1991); Hedaya, (1995) and Helal, et al., (1998). The obtained data for rats treated with ACR plus N.S. indicated that NS treatment nearly subverts the toxic effects of ACR on hematological picture and may protect the male rats from immunosuppressive effect of ACR.

On the other hand, it had been found that ACR and ACR plus NS produce a highly significant decrease in the rate of mitosis (Table 4) and (Fig.1) this results for ACR was in agreement with Adler, et al., (1993). They found that the progression of cell division was altered by Acrylamide. Table (5) showed that ACR and ACR plus NS induced highly significant effect on chromosomes structure and number. The different type of aberrations were tandam fusion (chromosome stickness) chromatide deletions, chromatid breaks, fragments and heteroploid cells (Fig.2). Treatment with Nigella sativa alone produces non significant effect on chromosome structure and numbers. Early studies of ACR on chromosomes demonstrated that, it had a genotoxic effect on different cells. Sega and Generoso (1990) reported DNA breakage in spermiogenic stages of the mouse after exposure to ACR. Adler et al., (1993) suggested that ACR might induce anaploidy in mammalian cells in vitro by interfering with proper functioning of the spindle fiber. Moreover, Tsuda et al., (1993) and Pacchierotti et al., (1994) observed different types of chromosomal aberrations induced by ACR.. They suggested that the mechanism of chromosomal changes mediated through the Alkylation of DNA-associated protamines. It was concluded that the Nigella sativa in such used dose level failed to ptotect the cells against the effect of ACR but, it produce insignificant adverse effects when injected alone.

Finally, the ultastructural changes was noticed only after two weeks from ACR treatment, the lesions in case of ACR were more severe than that treated with ACR plus NS. Electron micrograph showed that severe demylination of the sciatic nerve was a permanent alteration either in case of group III or group IV. Ultrastructurally, the degenerated nerve showed separation and disintegration of the lamellae of the myelin sheath, the degree of separation was mild focal in rats of group IV (Fig.3) or severe and diffuse in rats of group III (Fig.4) . The noticeable lesions in central nervous system (Brain) were only confirmed to rats given ACR (group III) (Fig.5). Other ultrastructural lesions for spinal cord were recorded in (Fig.6 and 7). Matsouka, et al., (1993) found that ACR treatment causes centeral-peripheral distal axonopathy, and inhibited creatine kinase activity in the brain and spinal cord which may play a role in the pathogenesis of distal degeneration in the central and peripheral nervous systems. These

obtained data regarding the neutrotoxicity of ACR is going hand in hand with finding of Abou-Donia, et al., (1993). They recorded that ACR produces centeral-peripheral distal axonopathy in humans and some animals species. Its neutrotoxicity is characterized by abnormal sensation, decreased motor strength, and ataxi.The most probable explanation for the neutroxicity of ACR may be contributed to inhibition of brain creatine kinase activity as well as reduction of GSH of the brain (Beiswanger et al., 1993).

From the aforemention results and discussion, it is concluded that ACR in dose of 0.05% in drinking water has hepato, geno and neutrotoxic effects as well as alterations in the hematological pictures. Nigella sativa seed extracts falls to protects the ACR treated animals completely against the adverse effects of ACR except for blood picture which improved it towards that of control group.

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