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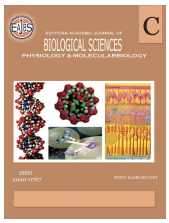

Nigella sativa Seeds Extract Ameliorates Toxicity Induced by Doxorubicin and Gamma Radiation in Rats

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ABSTRACT

Chemotherapy and radiotherapy are among the traditional methods used in cancer treatment and they cause toxicity to normal cells. The purpose of the present study is to determine the possible protective role of *Nigella sativa* seed (2 g/kg) extract as a potent antioxidant in improving the toxicity of doxorubicin (DOX) and/or gamma radiation in albino rats. The rats were injected with DOX (2.5 mg/kg, i.p.) and/or exposed the rats to irradiation (2 Gy, whole-body) weekly, for four consecutive weeks. The antioxidant treatments were used daily *via* oral gavages for two weeks protection period and during the experiment (4 weeks). The DOX and/or irradiated groups recorded a severe reduction of antioxidant parameters (superoxide dismutase, catalase and reduced glutathione) as well as increased thiobarbituric acid reactive substances, and changes of liver function parameters (transaminases, alkaline phosphatase, total protein and albumin as compared with control rats. Administration of *Nigella sativa* seed extract to DOX and/or irradiated rats indicates significantly enhanced the oxidative stress markers and liver function parameters as compared with DOX and/or irradiated rats.

In conclusion, *Nigella sativa* seed extract fruit extract used to decrease the bad side effects of chemotherapy and radiotherapy.

INTRODUCTION

The incidence and prevalence of cancer have been increasing in such a degree that it has become the second leading cause of death globally. In 2018 World Health Organization approved that cancer is a leading cause of death in many countries, accounting for 9.6 million deaths, approximately 70% of deaths from cancer occur in low- and middle-income countries, (WHO, 2018). Liver cancer is one of the most common cancers representing a leading cause of cancer-related mortality, with an increasing incidence worldwide, being responsible for about one million deaths every year (Lee *et al.*, 2005; Ferlay *et al.*, 2015)

Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy and targeted therapy (National Cancer Institute, 2018 & WHO, 2018).

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve symptoms. It works by damaging the DNA of cancerous tissue, killing it. To spare normal tissues (such as skin or organs, which radiation must pass through to treat the tumor), shaped radiation beams are aimed from multiple exposure angles to intersect at the tumor, providing a much larger dose there than in the surrounding, healthy tissue. As with chemotherapy, cancers vary in their response to radiation therapy (Tidy, 2017). Radiotherapy for the treatment of hepatocellular carcinoma causes severe bad side effects; including acute hepatitis and chronic fibrosis (Kuo *et al*., 2019). Ionizing radiation-induces oxidative stress demonstrated by oxidative damage in the liver (Shedid *et al.*, 2018), pancreas (Brodsky *et al*., 2002) subcellular membranous structures (Azab, 2007).

The reactive oxygen species (ROS) are highly active molecules containing oxygen that reacts with biological molecules and destroy the cells (Baatout *et al*., 2004). The increased ROS formation and decreased antioxidant protection caused oxidative stress to lead to cell death (Turrens, 2003). In addition, they are responsible for protein denaturation and impaired enzyme activity (Bashandy *et al*., 2014). Doxorubicin (DOX), also called adriamycin is an anthracycline antibiotic that has been used for a long time in the therapy of an array of human malignancies either alone or combined with other cytocidal agents (Giampieri *et al*., 2016; Mohebbati *et al*., 2016). DOX is a potent antibiotic used for the treatment of different tumors (Patil *et al*., 2008). The rats injected with doxorubicin and/or exposed to ionizing radiation-induced oxidative stress and several bad side effects as well as irreversible toxicity (Maity *et al*., 1994; Elsadek *et al*., 2017).

Nigella sativa is a member of the Ranunculaceae family. The seed is mostly used as a spice and extensively used in the traditional medicine of many countries (Sultan *et al*., 2015). *Nigella sativa* was commonly known in the Middle East as Habbatul Baraka or the 'seed of blessing' has curative potential as described in the Old Testament and by the prophet (Peace be upon him) himself (Gali-Muhtasib *et al.*, 2004), Abu Hurayrah (ra) narrates that Prophet Muhammad (Peace be upon him) said, *"Use this black seed regularly; because it is a remedy (cure) for every disease except death."* (Reference: Sahih Al-Bukhari 71:591, 592; Sahih Muslim 26: 5489).

Nigella sativa seed extract, fixed oil, and essential oil showed a wide spectrum of favorable biological activities, the most prominent being antioxidant (Entok *et al.*, 2014), anti-inflammatory (Majdalawieh & Fayyad, 2015), antiviral (Barakat *et al.*, 2013), antimicrobial (Sarwar & Latif, 2015), anti-mutagenic and hepatoprotective (John & Madusolumuo, 2017), antitumor activities (Linjawi *et al.*, 2015) and used in different forms to treat many diseases including asthma, hypertension, cough, bronchitis, headache, eczema, fever, dizziness and influenza (Entok *et al.*, 2014). Thymoquinone (TQ) is the bioactive phytochemical constituent of the seeds oil of *N. sativa.* In vitro and In Vivo research has thoroughly investigated the anticancer effects of TQ against several cancer cell lines and animal models (Majdalawieh *et al.*, 2017).

In light of the previous hypothesis, the present study aims to investigate the potential hepatoprotective effects of *Nigella sativa* seed Fruit extract, as potent antioxidants, in their recommended antioxidant doses, against doxorubicin

and gamma radiation-induced oxidative stress in Wistar rats.

MATERIALS AND METHODS

Doxorubicin (Dox):

Doxorubicin was purchased from EIMC united pharmaceuticals, Co. Egypt as Adricin® (doxorubicin hydrochloride). The doxorubicin injection dose in the present study was 2.5 mg/kg, i.p., weekly for four consecutive weeks (10 mg/kg body weight cumulative doses).

Irradiation (R):

The Canadian Gamma cell–40 (137Cs) used (National Center for Radiation Research and Technology (NCRRT), Nasr City, Egypt) to irradiated rats with a whole-body fractioned dose (2 Gy every week for four weeks up to 8 Gy cumulative doses). The dose rate at the time of the experiment was 0.45 Gy/min (4.44 min exposure times).

Plant Extract:

Preparation of *Nigella sativa* Seeds Alcoholic Extract:

Alcoholic extract of *Nigella sativa* seeds was prepared according to the method of Hadjzadeh *et al*. (2011) with a special modification. *N.S* seeds were obtained from a local market with a high degree of quality assurance. The seeds were cleaned under running tap water for 10 min, rinsed twice with distilled water and dried in an oven at 40 °C overnight until a constant weight was attained; then the seeds were powdered in an electrical grinder (blender) and stored at 5ºC until further use. Seed powder was extracted with a sufficient volume of 70% ethanol using a flask. Ethanol was evaporated at 40-50°C under reduced pressure and the yield of extract was freshly prepared by dissolved in isotonic saline (2 g of *N.S* extract (Shahid *et al*., 2017) suspended in 10 ml of normal saline Nacl 0.9%) and left a few minutes before administration (Radwan & Mohamed, 2018).

Experimental Animals:

The present study used 64 male Wistar albino rats weighing (120–130) g were obtained from the Animal Farm of the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt). The animals were housed in an animal facility that was maintained with a conditioned atmosphere at 25 ± 2°C and kept on standard diet-pellets (El-Nasr, Cairo, Egypt), and tap water. Animals were housed in metallic cages and maintained under standard conditions of temperature, humidity, and 12 hr light/dark cycle along the experimental period. Food and water were available throughout the experiment *ad libitum*. Rats were left to acclimatize for one week before starting the experiment.

Wistar albino rats (120–130 g) used 64 males at the beginning of this experiment. The rats were divided randomly and assigned into eight equal experimental groups (contains8 rats in each group) as the following:

Group I: (Control) rats of this group were neither treated nor irradiated and were provided with standard diet-pellets and drinking tap water *ad libitum* during the experiment for six weeks.

Group II: (*Nigella sativa*) comprised of normal rats have daily received an oral dose of 2 g/kg body weight of *N.S* alcoholic extract dissolved in saline via an oral gastric tube for six weeks.

Group III: (DOX) comprised of normal rats injected intra-peritoneally with four equal doses at (2.5 mg/kg body weight) of DOX for 4 times alternatively over a four weeks to make a total cumulative dosage of (10 mg/kg body weight), The first dosage was given on the seventh day from the beginning of the experiment (Fathy *et al*., 2017).

Group IV: (N.S+DOX) comprised of normal rats were intra-peritoneally injected with DOX as in group V and

administered 2 g/kg b.w/day of *N.S* orally for one week prior exposure to DOX. The administration of the *N.S.* was extended during the period of the experiment for six weeks.

Group V: (R group) comprised of normal rats were exposed to whole-body with four equal doses at (2 Gy body weight) of irradiation for 4 times alternatively over a four weeks to make a total cumulative dosage of (8 Gy body weight), The first dosage was given on the seventh day from the beginning of the experiment (Fathy *et al*., 2017).

Group VI: (N.S+R) comprised of normal rats were exposed to whole-body with irradiation as in group V and administered 2 g/kg b.w/day of *N.S* orally for one week prior exposure to Radiation. The administration of the *N.S.* was extended during the period of the experiment for six weeks.

Group VII: (DOX-R) comprised of normal rats injected intra-peritoneally with four equal doses of DOX and exposed to whole-body irradiation (DOX-R) for 4 times alternatively over four weeks, rats of this group were irradiated following 20h of DOX injection.

GroupVIII:(*N.S*+DOX)-R) comprised of normal rats injected intraperitoneally with four equal doses of DOX and exposed to whole-body irradiation (DOX-R) as in group VII and administered 2 g/kg b.w/day of *N.S* orally for one week prior exposure to DOX-R. The administration of the *N.S.* was extended during the period of the experiment for six weeks.

Collection and Preparation of Samples:

Blood samples were collected at the end of the experiment from each animal under anesthesia from the retro-orbital plexus using capillary tubes. Blood samples were collected and put into non-heparinized plain tubes, which were centrifuged at (4000 rpm) 1788g for 10 min. The serum samples were frozen at -80 °C for the following measurements.

After sampling, animals were sacrificed and livers were isolated, dissected out and washed with isotonic saline. Each tissue was homogenized in ice-cold phosphate (0.05 M - KCl, pH 7.4) buffer solution for 30 seconds twice to yield a 10% (w/v) by using (Heidolph, Diax 9000 apparatus) homogenizer. The homogenates were centrifuged under cooling at 4000 rpm for 20 min. The supernatants were subsequently aliquoted and stored at -80 °C until used.

Biochemical Study:

The liver tissue homogenates were used for the determination of hepatic thiobarbituric acid reactive substances (TBARS) (Yoshioka et al., 1979), and hepatic reduced glutathione (GSH) (Beutler et al., 1963). In addition, the activities of superoxide dismutase (SOD) (Kakkar *et al.,* 1984) and catalase (CAT) (Aebi, 1984) enzymes were estimated in the homogenates using kits from bio-diagnostic Co., Egypt.

The serum levels of transaminases (AST & ALT) (Bergmeyer et al., 1986), alkaline phosphatase (ALP) (Moss, 1982), total protein (TP) (Gornal et al., 1949) and albumin (Doumas et al., 1971) were estimated using kits from Egyptian Company for biotechnology spectrum, Egypt.

Statistical Analysis:

Statistical package for social sciences SPSS/PC computer program (version 20, USA) was used for the statistical analysis of the present results. The results were analyzed using analysis of variance (ANOVA) one-way test followed by the least significant difference test for multiple comparisons. Differences were considered statistically significant at $p<0.05$. Data are summarized as mean ± standard error.

RESULTS

Nigella sativa and TQ have been found to be effective in protecting liver toxicity induced by DOX treatment. The represented data

of DOX and radiation intoxicated rats showed significant raises ($P<0.05$) in serum ALAT, ASAT and ALP enzyme activity as compared with the control group (Table 1). While a significantly decreased ($P<0.05$) might be restored to normal levels in rats pre-co-treated with N.S extract in combination with DOX and radiation as compared with the corresponding value of DOX and radiation groups.

The percentage of change from serum total protein and albumin in the groups treated with DOX and radiation showed a significant decrease when compared with the corresponding value of the control group. On the other hand, the percentage change of serum total protein and albumin in treated rats with N.S extract in combination with DOX and radiation showed a significant increase as compared with the corresponding value of DOX and radiation groups.

The percentage of change from an antioxidant and oxidative biomarker of liver homogenate in the groups treated with DOX and radiation alone or with combination showed a significant decrease in parameters of SOD, CAT, and GSH when compared with a corresponding value of control group (Table 2). While an increased in MDA and NO in rats treated with DOX and radiation alone or with the combination when compared with the corresponding value of the control group. On the other hand, the percentage change of CAT, SOD, and GSH in rats treated with N.S extract in combination with DOX and radiation showed significant increase as compared with the corresponding value of DOX and radiation groups and also a significant decreased in MDA and NO in rats treated with N.S extract in combination with DOX and radiation when compared with the corresponding value of DOX and radiation groups.

Table 1: Serum of liver function test in non-treated rats and rats exposed to DOX and/or Radiation treated with N.S. for 6 weeks.

Parameter / Groups	Liver function test									
	ALAT (U / L)		ASAT (U / L)		ALP (U / L)		Total protein (g/dl)		Albumin (g/dl)	
	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change
Group I (Control)	65.52± 1.12^{a}		70.4±1.9^{a}		115±1.3^{a}		6.5±0.19^{a}		4.22±0.13^{a}	
Group II N.S	63.9±0.9^{a}	-2.46	70.23±2.56^{a}	-0.252%	113.2±.1.3^{a}	-1.62	6.6±0.14^{a}	1.79	4.27±0.21^{a}	1.21%
Group III DOX	118.1±3.16^{b}	80.27	98.53±3.97^{b}	40.29%	172.1±4.3^{b}	49.78%	5.2±0.04^{b}	--20.47%	2.87±0.08^{b}	-31.99%
Group IV N.S+DOX	79.07±0.7^{c,e}	20.67	88.6±0.87^{c,d}	25.84%	140.7±2.8^{c}	22.34%	5.9±0.12^{c}	-9.94%	3.42±0.16^{c}	-18.99%
Group V R	105.5±3.63^{d}	61.02	94.6±3.17^{b,c}	34.36%	162.6±4.3^{d}	41.34%	5.3±0.12^{b,d}	-17.95%	3.15±0.04^{b,c}	-25.39%
Group VI (N.S+R)	75.94±1.37^{c}	14	77±2.56^{e}	9.36%	125.4±2.2^{e}	9.04%	6.2±0.18^{c}	-5.22%	3.56±0.1^{c}	-15.76%
Group VII DOX+R	134.3±3.2^{f}	105	112.42±1.34^{f}	59.66%	177.7±2.4^{b}	54.46%	4.6±0.21^{e}	-29.26%	3.87±0.16^{b}	-31.93%
Group VIII N.S+(DOX+R)	83.1±3.6^{e}	26.8	83.03±1.06^{d,e}	17.92%	150±6.6^{f}	30.43%	5.7±0.19^{c,d}	-12%	3.49±0.19^{c}	-17.36%

N.S = *Nigella sativa*; **R** = *Radiation* and **DOX** = Doxorubicin
Data are expressed as mean ± standard error Significant start from ($P<0.05$).
Percentage changes (%) are calculated by comparing treated groups with normal control group.

Table 2: Liver antioxidant and bio-marker test in non-treated rats and rats exposed to DOX and/or Radiation treated with N.S. for 6 weeks.

Parameter / Groups	Liver antioxidant bio-marker									
	TBARS (nmole/ g wet tissue)		NO (µmol /g wet tissue)		GSH (mg /g wet Tissue)		CAT (u/mg protein)		SOD (u/mg protein)	
	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change	Mean ±SE	%change
Group I (Control)	196±1.58^{a}		43.17±0.26^{a}		2.94±.02^{a}		1.56±.02^{a}		74.77±.73^{a}	
Group II N.S	193±1.44^{a}	-1.53%	44.24±0.14^{a}	2.48%	2.98±.03^{a}	1.36	1.54±.02^{a,b}	-1.28%	75.37±1.1^{a}	0.8%
Group III DOX	224±1.94^{b}	14.28%	64.76±1.53^{b}	50%	2.39±.03^{b,d}	-18.7%	1.25±.02^{c,d}	--19.87%	65.79±.49^{b}	-12%
Group IV N.S +DOX	213±0.93^{c}	8.67%	64.52±2.94$^{c.}$	26.29%	2.65±.03^{c}	-9.86%	1.45±.03^{a,b}	-7.05%	70.54±.48^{c}	-5.66%
Group V R	221±1.52^{b}	12.75%	77.9±1.57^{d}	80.45%	2.47±.03^{b}	-15.99%	1.34±.03^{b,c}	-14.1%	66.45±.47^{b}	-11.13%
Group VI (N.S+R)	213±1.12^{c}	8.67%	69.25±1.21^{b}	60.41%	2.74±.03^{c}	-6.8%	1.5±.01^{a,b}	-3.85%	69.82±.45^{c}	-6.62%
Group VII DOX+R	238±1.6^{d}	21.43%	88.3±2.49^{e}	104.54%	2.23±.03^{e}	-24.15%	1.06±.18^{d}	-32.05%	60.53±.39^{d}	-19.05%
Group VIII N.S +(DOX+ R)	231±2.64^{e}	17.86%	71.7±1.63^{f}	66.09%	2.31±.03^{d,e}	-21.43%	1.15±.02^{c,d}	-26.28%	64.46±.53^{b}	-13.79%

N.S = *Nigella sativa*; **R** = *Radiation* and **DOX** = Doxorubicin
Data are expressed as mean ± standard error Significant start from (P<0.05).
Percentage changes (%) are calculated by comparing treated groups with normal control group.

DISCUSSION

The exposure to ionizing radiation is known to induce oxidative stress through the generation of ROS resulting in an imbalance of the pro-oxidant and antioxidant activities ultimately resulting in cell death (Srinivasan *et al*., 2006**)**. ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy and experimentation (Fang *et al*., 2002; Kaya *et al.,* 2009). These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes (Schinella *et al.,* 2002; Cadet *et al*., 2004). A product of lipid peroxidation such as MDA has the ability to interact with and alter macromolecules, possibly resulting in diseases (Box & Maccubbin, 1997).

The increased MDA level from the results analysis that observed in serum liver tissue showed an elevation of MDA in the DOX and/or radiation treated groups, this elevation was significantly decreased as compared with other treated groups which could be attributed to the peroxidation of membrane's lipid resulted in cellular structure modifications and contributing the development of oxygen radical-mediated tissue damage (Leyko & Bartoz, 1986; El-zawahry *et al.*, 2016).

These results are in accordance with El-Missiry *et al*., (2007) who reported that the exposure of rats to γ-radiation (2 Gy and 4 Gy) induced a significant increase in liver TBARS concentration, a lipid peroxidation index (Hamza & Shaaban, 2019).

The results are in agreement with those of several authors who reported that oxidative stress leads to lipid peroxidation, which is the result of an interaction between free radicals of diverse origin and unsaturated fatty acids typically in membrane lipids. The net result of these events is the accumulation of a variety of toxic lipid peroxides and malondialdehyde (MDA). The level of tissue MDA is reported to be a reliable marker of lipid peroxidation (Hamza *et al*., 2008; Patel *et al.*, 2010).

Elevations in MDA level suggest that oxidative stress due to free-radical damage; this might occur due to two different ways of free radical formation by DOX have been described, the first implicates the formation of a semiquinone free radical which yields superoxide

radicals (Singal *et al.,* 2000) and the second way produces Fe^{2+}-DOX complex (De Beer *et al.,* 2001) that can reduce oxygen to hydrogen peroxide and other active species.

On the other hand, rats were administered N.S extract orally plus DOX showed a significant decrease in lipid peroxidation status restored to normal levels when compared with the corresponding values of DOX group. Therefore; the hepatoprotective activity exhibited by *N. sativa* extract might be due to the anti-oxidative nature of the plant (El-zawahry *et al.,* 2016).

The results could be interpreted with Meral *et al.* (2001) who reported that the treatment of CCl_4 exposed rats with N.S was able to protect the liver from damage by decreased MDA levels in their study. Also, these results are in accordance with the finding of Yesmin *et al.* (2013) reported that the pre-treated with the extract of N.S in a mixture with paracetamol treated group shows decreased the elevated levels of hepatic MDA while it was significantly higher. This decline might be due to less lipid peroxidation or less oxidative stress.

Superoxide dismutase (SOD) is the only enzyme known to utilize free radicals as a substrate; also it catalyzes the reduction of $O_2^{\cdot -}$ radical to H_2O_2 (Balin & Allen, 1986). The results revealed a significant decrease in SOD activity recorded in the liver tissue of irradiated rats. These decreases might be due to overexpression of the enzyme as one of the self-defense mechanisms against oxidative stress where SOD system, constitutes the first line of defense against the deleterious effects of ROS (Craven *et al*., 2001). The results are consistent with Guo *et al*., (2003), who stated that exposure to fractionated ionizing radiation induces the expression of endogenous SOD.

The results of liver CAT, reduced GSH and SOD activities in DOX and/or γ-irradiation treated group observed a significant decrease when compared to the corresponding values in control group (Mete *et al*., 2016; Hamza & Shaaban, 2019) as well as the obtained data observed a significant increase in the groups were administrated N.S extract in a combination with DOX and/or γ-irradiation showed restored to normal levels when compared to the corresponding values in DOX and/or γ-irradiation treated group (Bhandari *et al*., 2012; El-yamany *et al.,* 2016).

Normalization in SOD activity suggests that pretreatment with N. S extract may have the ability to prevent the deleterious effects induced by free radicals (Aniss *et al*., 2014). The results are in agreement with Meral *et al*. (2001); Mohebbati *et al*. (2016) who reported that the treatment with N.S able to protect the liver from damage by increased CAT, reduced GSH and SOD.

The obtained data of serum ALAT, ASAT and ALP activities revealed a significant elevation in DOX and/or γ-irradiation intoxicated rats as compared to the control group. Serum aminotransferases (ALAT & ASAT) and ALP are cytosolic enzymes located in hepatocytes and an increase in their activities reflecting the increase in plasma membrane permeability which in turn are associated with cell death (Gralnek *et al*., 2000; Davies and Azeez, 2016; Hamza & Shaaban, 2019).

DOX-induced hepatotoxicity might be due to the excessive formation of free radicals formed during its detoxification in the hepatocytes smooth endoplasmic reticulum by cytochrome P450. The higher activities of ASAT, ALAT, and ALP may be a result of drastic conditions which caused by the toxic activity of DOX accumulations in the liver and in turn, this might provoke cellular destruction or increase the permeability of hepatic cells (Wilhelm *et al.,* 1996; Mcnulty & Taylor, 1999). Administration of DOX significantly

increased the injury marker enzymes of the liver, such as SGPT and SGOT our findings are in good agreement with those of previous studies (Rašković *et al*., 2011; Mete *et al*., 2016; Ibrahim *et al.,* 2018).

The results are in accordance with Saada & Azab, (2001) who reported that the increase in serum enzymes (ALAT & ASAT) activities might be due to the loss of cellular functional integrity of hepatocytes membrane resulted from membrane lipids peroxidation by radiation-induced free radical generation where the peroxidation of biological membrane's lipids by ROS results in structural changes of membranes and release of their contents.

On the other hand serum ALAT, ASAT and ALP activities showed a significant decrease in the groups treated with N.S in combination with DOX and/or γ-irradiation when compared with the corresponding value of DOX and/or γ-irradiation. This reduction suggests that the used extract might be considered as a promising beneficial therapeutic agent against hepatoxicity induced by DOX and/or γ-irradiation might be due to the presence of antioxidant actions in their active constituents. This reduction upon the protective effect of those herbs extracts which return to normal serum values; the role of protection may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration (Ameen *et al.*, 2011; Dollah *et al.,* 2013; Hashem *et al*., 2018; Erisgin *et al*., 2019).

The results are in agreement with the hypothesis of Hozayen *et al.* (2014) who recorded that, the evidence is available demonstrating a significant increase of ALAT, ASAT and ALP activities in rats were administrated DOX and the pre-treatment with rutin, hesperidin and their mixture successfully ameliorated the elevated activities.

Cikman *et al*. (2014) found that NSO caused a decrease in oxidative stress markers in liver tissue when administered orally 1 g/kg/day for 10 days before radiotherapy.

Albumin values are associated with the function of hepatic cells**.** The result of serum total protein and albumin concentrations in DOX and/or γ-irradiation intoxicated rats showed a significant decrease when compared with the control group. The results are in agreement with (Assayed, 2010; Hozayen *et al*., 2014; Davies & Azeez, 2016) who reported that serum albumin level was significantly decreased in DOX or γ-irradiation intoxicated rats.

On the other hand, the represented data revealed that total protein and albumin concentrations in the serum showed a significant increase in rats from groups pre-co-treated with N.S in a combination with DOX and/or γ-irradiation restored to normal levels when compared with the corresponding values of DOX and/or γ-irradiation group. This indicates that N.S extract improved the cellular functional by maintaining the integrity of hepatocytes membrane resulted from membrane lipids peroxidation by DOX induced free radical generation where the peroxidation of biological membrane's lipids by ROS results in structural changes of membranes and generated ROS which exceeds antioxidants capability of free radical scavengers (Türkdoğan *et al*., 2001; Mahmoud *et al*., 2002; Alenzi *et al*., 2010; Al Ameen *et al*., 2011; Paul *et al*., 2019).

Shahid *et al*. (2018) examined the enzymes in the NSO (2 ml/kg, orally) and TQ (1.5 mg/kg, orally) (NS derivative) administration for 14 days before cisplatin exposure and the following 6-day administration during chemotherapy (20 days in total). It was reported that both substances were protective against negative effects of cisplatin on intestines, but NSO proved to be more effective than TQ.

Conclusion:

The results of this work demonstrate that using of chemotherapeutic drugs such as DOX in the treatment of a variety of human cancers leads to significant liver damage while oral administration of N.S and their mixture extract provide a significant protective role against toxicity caused by DOX. Collectively, these results could demonstrate that supplementation rich with N.S could be used in combination with DOX to protect against hepatotoxicity, avoiding the need to take other medications, and improving the patient's quality of life.

REFERENCES

Aebi, H. (1984): Catalase in vitro. In Methods in enzymology (Vol. 105, pp. 121-126). Academic Press.

Alenzi, F. Q., El-Bolkiny, Y. E. S., & Salem, M. L. (2010): Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. British journal of biomedical science, 67(1), 20-28.

Ameen, N. M. A., Altubaigy, F., Jahangir, T., Mahday, I. A., Mohammed, E. A., & Musa, O. A. A. (2011): Effect of *Nigella sativa* and bee honey on pulmonary, hepatic and renal function in Sudanese in Khartoum state. Journal of Medicinal Plants Research, 5(31), 6857-6863.

Aniss, H. A.; Said, A. E. M.; El Sayed, I. H. and Adly, C. (2014): Amelioration of adriamycin-induced cardiotoxicity by *Salsola kali* aqueous extract is mediated by lowering oxidative stress. Redox Report, 19(4): 170-178.

Assayed, M. E. (2010): Radioprotective effects of black seed (*Nigella sativa*) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats. Immunopharmacology and immunotoxicology, 32(2), 284-296.

Azab, K. S. (2007): Modulation of radiation injuries in rats receiving multiple doses of *Aloe Vera*. Egyptian Journal of Radiation Sciences and Applications, 20(1), 17-28.

Baatout, S., Derradji, H., Jacquet, P., Ooms, D., Michaux, A., & Mergeay, M. (2004): Enhanced radiation-induced apoptosis of cancer cell lines after treatment with resveratrol. International journal of molecular medicine, 13(6), 895-902.

Balin, A. K. and Allen, R. G. (1986): Mechanisms of biologic aging. Dermat. Clin., 4:347-358.

Barakat, E. M. F., El Wakeel, L. M., & Hagag, R. S. (2013): Effects of *Nigella sativa* on outcome of hepatitis C in Egypt. World journal of gastroenterology: WJG, 19(16), 2529.

Bashandy, M. A., Hasan, H. F., & Fathy, A. H. (2014): Protective and therapeutic effects of olive oil and *Ficus carica* as natural antioxidants on some biochemical parameters in liver of γ-irradiated male albino rats. Al Azhar Bulletin of Science, 25(1), 1-16.

Bergmeyer, H. U., Herder, M., & Ref, R. (1986): International Federation of Clinical Chemistry (IFCC). J. Clin. Chem. Clin. Biochem, 24(7), 497-510.

Beutler, E. (1963): Improved method for the determination of blood glutathione. *J. lab. clin. Med., 61, 882-888.*

Bhandari, U.; Pathan, R. and Nag, T. (2012): Anti-apoptotic potential of gymnemic acid phospholipid complex pretreatment in Wister rats with experimental cardiomyopathy. Indian J of Exp Biol., 50: 117-127.

Box, H.C. and Maccubbin, A.E. (1997): Lipid peroxidation and

DNA damage. Nutrition, 13 (10): 920–921.

Brodsky, S. V., Gao, S., Li, H., & Goligorsky, M. S. (2002). Hyperglycemic switch from mitochondrial nitric oxide to superoxide production in endothelial cells. *American Journal of Physiology-Heart and Circulatory Physiology*, *283*(5), H2130-H2139.

Cadet, C.; Metz, J.A.J. and Klinkhamer, P.G.L. (2004): Size and the not-so-single sex: Disentangling the effects of size and budget on sex allocation in hermaphrodites. *Am. Nat*., 164: 779-792.

Cikman, O., Ozkan, A., Aras, A. B., Soylemez, O., Alkis, H., Taysi, S., & Karaayvaz, M. (2014): Radioprotective effects of *Nigella sativa* oil against oxidative stress in liver tissue of rats exposed to total head irradiation. Journal of Investigative Surgery, 27(5), 262-266.

Craven, P. A.; Melhem, M. F.; Phillips, S. L. and DeRubertis, F. R. (2001): Overexpression of Cu21/Zn21 superoxide dismutase protects against early diabetic glomerular injury in transgenic mice. Diabetes, 50: 2114-2125.

Davies, O., & Azeez, O. (2016): Modulatory effects of guava extract on Adriamycin (Doxorubicin) induced toxicity in Wistar Rats. Glob. Vet, 16, 31-36.

De Beer, E. L.; Bottone, A. E. and Voest, E. E. (2001): Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. European journal of pharmacology, 415(1): 1-11.

Dollah, M. A., Parhizkar, S., Latiff, L. A., & Hassan, M. H. B. (2013): Toxicity effect of *Nigella sativa* on the liver function of rats. Advanced pharmaceutical bulletin, 3(1), 97.

Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971): Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta, 31(1), 87-96.

El-Missiry, M. A.; Fayed, T. A.; El-Sawy, M. R. and El-Sayed, A. A. (2007): Ameliorative effect of melatonin against gamma-irradiation induced oxidative stress and tissue injury. Ecotoxicology and Environmental Safety, 66: 278–286.

Elsadek, B., Mansour, A., Saleem, T., Warnecke, A., & Kratz, F. (2017): The antitumor activity of a lactosaminated albumin conjugate of doxorubicin in a chemically induced hepatocellular carcinoma rat model compared to sorafenib. Digestive and Liver Disease, 49(2), 213-222.

El-yamany, I., Kholoussi, N., Raouf, H. A., Ibrahim, D. F., & Hussein, A. S. (2016): *Nigella sativa* and *Aloe vera* protection against doxorubicin hepatotoxicity. J Exp Appl Animal Sci (Sl), 2(1), 10-22.

Entok, E., Ustuner, M. C., Ozbayer, C., Tekin, N., Akyuz, F., Yangi, B. & Gunes, H. V. (2014): Anti-inflammatuar and anti-oxidative effects of *Nigella sativa* L.:18 FDG-PET imaging of inflammation. Molecular biology reports, 41(5), 2827-2834.

Erisgin, Z., Atasever, M., Cetinkaya, K., Dizakar, S. Ö. A., Omeroglu, S., & Sahin, H. (2019): Protective effects of *Nigella sativa* oil against carboplatin-induced liver damage in rats. *Biomedicine & Pharmacotherapy*, *110*, *742-747*.

Fang, Y.; Yang, S. and Wu, G. (2002): Free radicals, antioxidants, and nutrition. Nutrition, 18: 879–887.

Fathy, A. H., Bashandy, M. A., Mansour, A. M., Azab, K. S., & Bashandy, S. A. (2017): Hepatoprotective effects of olive oil with fig and date-palm fruit extracts in rats treated with doxorubicin and gamma radiation. Advances in Pharmaceutical and Ethnomedicines, 5(1), 8-15.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., ... & Bray, F. (2015): Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer, 136(5), E359-E386.

Gali-Muhtasib, H. U.; Abou Kheir, W. G.; Kheir, L. A.; Darwiche, N. and Crooks, P. A. (2004): Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. Anticancer Drugs, 15: 389-99.

Giampieri, F., Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernandez, T. Y., Afrin, S., Bompadre, S. & González-Paramás, A. M. (2016): Strawberry consumption alleviates doxorubicin-induced toxicity by suppressing oxidative stress. Food and chemical toxicology, 94, 128-137.

Gornal, A. C., Bardawill, C. J., & David, M. M. (1949): Protein–Biuret colorimetric method. J. Biol. Chem, 177, 751.

Gralnek, I. M., Hays, R. D., Kilbourne, A., Rosen, H. R., Keeffe, E. B., Artinian, L., ... & Martin, P. (2000): Development and evaluation of the liver disease quality of life instrument in persons with advanced, chronic liver disease the LDQOL 1.0. The American journal of gastroenterology, 95(12), 3552.

Guo, G.; Yan-Sanders, Y.; Lyn-Cook, B. D.; Wang, T.; Tamae, D.; Ogi, J.; Khaletskiy, A.; Li, Z.; Weydert, C.; Longmate, J. A.; Huang, T. T.; Spitz, D. R.; Oberley, L. W. and Li1, J. J. (2003): Manganese superoxide dismutase-mediated gene expression in radiation-induced adaptive responses. Molecular and Cellular Biology, 23: 2362–2378.

Hadjzadeh, M. A., Rad, A. K., Rajaei, Z., Tehranipour, M., & Monavar, N. (2011): The preventive effect of N-butanol fraction of *Nigella sativa* on ethylene glycol-induced kidney calculi in rats. Pharmacognosy magazine, 7(28), 338-343.

Hamza, A., Amin, A. and Daoud, S. (2008): The protective effect of a purified extract of *Withania somnifera* against doxorubicin-induced cardiac toxicity in rats. Cell biology and toxicology, 24(1): 63-73.

Shaaban, A. M. M., & Hamza, R. G. (2019). Studying the Ameliorative Effect of Bee Venom Against Damage and Inflammation Induced in Gamma-Irradiated Rats. *Arab Journal of Nuclear Sciences and Applications*, *52*(1), 178-184.

Hashem, M. A., Mohamed, W. A., & Attia, E. S. (2018): Assessment of protective potential of *Nigella sativa* oil against carbendazim-and/or mancozeb-induced hematotoxicity, hepatotoxicity, and genotoxicity. Environmental Science and Pollution Research, 25(2), 1270-1282.

Hozayen, W.; Abou Seif, H. and Amin, S. (2014): Protective Effects of Ruitn and / or Hesperidin against Doxorubicin-Induced Hepatotoxicity. International Journal of Clinical Nutrition, 2(1): 11-17.

Ibrahim, H. G., Attia, N., Fatma El Zahraa, A. H., & El Heneidy, M. A. (2018): Cerium oxide nanoparticles: In pursuit of liver protection against doxorubicin-

induced injury in rats. Biomedicine & Pharmacotherapy, 103, 773-781.

John, P. W., & Madusolumuo, M. A. (2017): *Nigella sativa* aqueous seed extract ameliorates cadmium-induced weight loss and anaemia in rats. Innovat International Journal of Medical & Pharmaceutical Sciences, 2(1):130-132.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984): A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984 Apr;21 (2):130-2

Kaya, C.; Ashraf, M.; Sonmez, O.; Aydemir, S.; Tuna, A. L.; and Cullu, M. A. (2009): The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. Scientia Horticulturae., 121(1): 1-6.

Kuo, T. H., Kuo, Y. H., Cho, C. Y., Yao, C. J., Lai, G. M., & Chuang, S. E. (2019): Protective Effect of *Antrodia cinnamomea* Extract against Irradiation-Induced Acute Hepatitis. International journal of molecular sciences, 20(4), 846.

Lee, S. E.; Steiner, M. and Lin, R. I. (2005): Thiallyl compounds: potent inhibitors of cell proliferation. Bio-chim Biophys Acta., 122: 73-77.

Leyko, W. and Bartoz, G. (1986): Membrane effects of ionizing radiation and hyperthermia. Int. J. Radiat. Biol., 49: 743–770.

Linjawi, S. A., Khalil, W. K., Hassanane, M. M., & Ahmed, E. S. (2015): Evaluation of the protective effect of *Nigella sativa* extract and its primary active component thymoquinone against DMBA-induced breast cancer in female rats. Archives of medical science: AMS, 11(1), 220.

Mahmoud, M. R., El-Abhar, H. S., & Saleh, S. (2002): The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. Journal of ethnopharmacology, 79(1), 1-11.

Maity, A., McKenna, W. G., & Muschel, R. J. (1994): The molecular basis for cell cycle delays following ionizing radiation: a review. Radiotherapy and oncology, 31(1), 1-13.

Majdalawieh, A. F., & Fayyad, M. W. (2015): Immunomodulatory and anti-inflammatory action of *Nigella sativa* and thymoquinone: A comprehensive review. International immunopharmacology, 28(1), 295-304.

Majdalawieh, A. F., Fayyad, M. W., & Nasrallah, G. K. (2017): Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*. Critical reviews in food science and nutrition, 57(18), 3911-3928.

McNulty, T. J. and Taylor, C. W. (1999): Extracellular heavy metal ions stimulate Ca2+ mobilization in epatocytes. J. Biochem., 339: 555-561.

Meral, I., Yener, Z., Kahraman, T., & Mert, N. (2001): Effect of Nigella sativa on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. Journal of Veterinary Medicine Series A, 48(10), 593-599.

Mete, R., Oran, M., Topcu, B., Oznur, M., Seber, E. S., Gedikbasi, A., & Yetisyigit, T. (2016): Protective effects of onion (*Allium cepa*) extract against doxorubicin-induced hepatotoxicity in

rats. Toxicology and industrial health, 32(3), 551-557.

Mohebbati, R., Shafei, M. N., Soukhtanloo, M., Roshan, N. M., Rad, A. K., Anaeigoudari, A., & Beheshti, F. (2016): Adriamycin-induced oxidative stress is prevented by mixed hydro-alcoholic extract of *Nigella sativa* and *Curcuma longa* in rat kidney. Avicenna journal of phytomedicine, 6(1), 86.

Moss, D. W. (1982): Alkaline phosphatase isoenzymes. Clinical chemistry, 28(10), 2007-2016.

National Cancer Institute (26 February 2018). "Targeted Cancer Therapies". Www.cancer.gov. Retrieved 28 March 2018.

Patel, V., Upaganlawar, A., Zalawadia, R., & Balaraman, R. (2010): Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: a biochemical, electrocardiographic and histoarchitectural evaluation. European journal of pharmacology, 644(1-3), 160-168.

Patil, R. R., Guhagarkar, S. A., & Devarajan, P. V. (2008): Engineered nanocarriers of doxorubicin: a current update. Critical Reviews™ in Therapeutic Drug Carrier Systems, 25(1).

Paul, J., Nasiruddin, M., Khan, I. A., Khan, R. A., & Arif, S. H. (2019): Therapeutic Effect of *Nigella sativa* Oil in Hepatotoxicity Induced by Isoniazid in Rats. Indian Journal of Pharmaceutical Education and Research, 53(2), 242-249.

Radwan, R. R., & Mohamed, H. A. (2018): *Nigella sativa* oil modulates the therapeutic efficacy of mesenchymal stem cells against liver injury in irradiated rats. Journal of Photochemistry and Photobiology B: Biology, 178, 447-456.

Rašković, A., Stilinović, N., Kolarović, J., Vasović, V., Vukmirović, S., & Mikov, M. (2011): The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules, 16(10), 8601-8613.

Saada, H.N. and Azab, Kh.Sh. (2001): Role of lycopene in recovery of radiation induced injury to mammalian cellular organelles. Pharmazie 56: 239–241.

Sarwar, A., & Latif, Z. (2015): GC–MS characterisation and antibacterial activity evaluation of *Nigella sativa* oil against diverse strains of Salmonella. Natural product research, 29(5), 447-451.

Schinella, G. R.; Tournier, H. A.; Prieto, J. M.; Mordujovich de Buschiazzo, P. and Rios, J. L. (2002): Antioxidant activity of anti-inflammatory plant extracts. Life Sci., 70:1023–1033.

Shahid, F., Farooqui, Z., Khan, A. A., & Khan, F. (2018): Oral *Nigella sativa* oil and thymoquinone administration ameliorates the effect of long-term cisplatin treatment on the enzymes of carbohydrate metabolism, brush border membrane, and antioxidant defense in rat intestine.Naunyn Schmiedeberg's archives of pharmacology, 391 (2), 145-157.

Shahid, F., Farooqui, Z., Rizwan, S., Abidi, S., Parwez, I., & Khan, F. (2017): Oral administration of *Nigella sativa* oil ameliorates the effect of cisplatin on brush border membrane enzymes, carbohydrate metabolism and antioxidant system in rat intestine. Experimental and toxicologic pathology, 69(5), 299-306.

Singal, P. K.; Li, T.; Kumar, D. Danelisen, I. and Iliskovic, N. (2000): Doxorubicin-induced heart- failure: mechanism and modulation. Mol. Cell. Biochem., 207(1-2): 77-85.

Srinivasan, M.; Sudheer, AR.; Pillai, K. R.; Kumar, P. R.; Sudhakaran, P. R. and Menon, V. P. (2006): Influence of ferulic acid on gamma radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes. Toxicology, 228(2-3): 249-58.

Sultan, M. T., Butt, M. S., Karim, R., Ahmad, A. N., Ahmad, R. S., & Ahmad, W. (2015): *Nigella sativa* fixed and essential oil improves antioxidant status through modulation of antioxidant enzymes and immunity. Pakistan journal of pharmaceutical sciences, 28(2).

Tidy C. "Radiotherapy". Patient UK. Archived from the original on 9 July (2017): Last Checked: 23 December 2015

Türkdoğan, M. K., Ağaoğlu, Z., Yener, Z., Sekeroğlu, R., Akkan, H. A., & Avci, M. E. (2001): The role of antioxidant vitamins (C and E), selenium and *Nigella sativa* in the prevention of liver fibrosis and cirrhosis in rabbits: new hopes. *DTW.* Deutsche tierarztliche Wochenschrift, 108(2), 71-73.

Turrens, J. F. (2003): Mitochondrial formation of reactive oxygen species. J. Physiol., 552: 335–344.

World Health Organization. 12 September 2018. Retrieved 19 December 2018.

Wilhelm, M.; Jaeger, D. E.; Schall-Cablitz, H.; Hafner, D. and Idel, H. (1996): Hepatic clearance and retention of aluminum: studies in the isolated perfused rat liver. Toxicol. Lett., 89 (3): 257-263.

Yesmin, F.; Rahman, Z.; Dewan, J. F.; Helalia, A. M.; Rahman, N. I.; Alattraqchi, A. G.; Ahmed, A.; Yousuf, R.; Salam, A and Haque, M. (2013): Hepato-protective role of the aqueous and n.hexane extracts of *Nigella sativa linn*. in rats. Asian J Pharm Clin Res., 6(3): 0974-2441.

Yoshioka, T., Kawada, K., Shimada, T., & Mori, M. (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American journal of obstetrics and gynecology, 135 (3),372-376.