
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 cytotoxic 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
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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 5°C 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 (contains 8 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.

**Group VIII:** (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 biodiagnostic 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>ALAT (U/L) Mean ±SE</th>
<th>% change</th>
<th>ASAT (U/L) Mean ±SE</th>
<th>% change</th>
<th>ALP (U/L) Mean ±SE</th>
<th>% change</th>
<th>Total protein (g/d) Mean ±SE</th>
<th>% change</th>
<th>Albumin (g/d) Mean ±SE</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>ALAT</td>
<td>65.52 ± 1.13</td>
<td></td>
<td>70 ± 1.9</td>
<td></td>
<td>115 ± 1.3</td>
<td></td>
<td>6.54 ± 0.15</td>
<td></td>
<td>4.22 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Group II N.S</td>
<td>ALAT</td>
<td>63 ± 0.9</td>
<td>-2.46</td>
<td>70 ± 2.66</td>
<td>0.252%</td>
<td>113 ± 2.1</td>
<td>-1.62</td>
<td>6.64 ± 0.14</td>
<td>1.79</td>
<td>4.27 ± 0.21</td>
<td>1.21%</td>
</tr>
<tr>
<td>Group III DOX</td>
<td>ALAT</td>
<td>118 ± 3.16</td>
<td>80.27</td>
<td>98.22 ± 3.97</td>
<td>40.29%</td>
<td>172 ± 4.2</td>
<td>49.78%</td>
<td>9.26 ± 0.4</td>
<td>-20.47%</td>
<td>2.87 ± 0.09</td>
<td>-31.90%</td>
</tr>
<tr>
<td>Group IV N.S+DOX</td>
<td>ALAT</td>
<td>79.97 ± 0.74</td>
<td>20.67</td>
<td>88.6 ± 0.87</td>
<td>25.84%</td>
<td>140 ± 2.8</td>
<td>22.34%</td>
<td>9.5 ± 0.12</td>
<td>-8.94%</td>
<td>3.3 ± 0.10</td>
<td>-18.98%</td>
</tr>
<tr>
<td>Group V R</td>
<td>ALAT</td>
<td>105.5 ± 3.45</td>
<td>61.02</td>
<td>94.6 ± 3.17</td>
<td>34.36%</td>
<td>162 ± 4.3</td>
<td>41.34%</td>
<td>5.3 ± 0.12</td>
<td>-17.85%</td>
<td>3.1 ± 0.04</td>
<td>-25.39%</td>
</tr>
<tr>
<td>Group VI N(S)+R</td>
<td>ALAT</td>
<td>71.94 ± 1.37</td>
<td>14</td>
<td>77.26 ± 3.6</td>
<td>9.36%</td>
<td>125 ± 2.2</td>
<td>9.04%</td>
<td>6.24 ± 0.18</td>
<td>-5.22%</td>
<td>3.56 ± 0.11</td>
<td>-15.76%</td>
</tr>
<tr>
<td>Group VII DOX+R</td>
<td>ALAT</td>
<td>134.92 ± 2.4</td>
<td>105</td>
<td>112.42 ± 1.3</td>
<td>59.66%</td>
<td>177 ± 2.0</td>
<td>54.46%</td>
<td>4.66 ± 0.21</td>
<td>-29.26%</td>
<td>3.57 ± 0.10</td>
<td>-31.93%</td>
</tr>
<tr>
<td>Group VIII N.S+(DOX+R)</td>
<td>ALAT</td>
<td>13.3 ± 3.8</td>
<td>26.1</td>
<td>83.01 ± 0.69</td>
<td>17.92%</td>
<td>150 ± 6.6</td>
<td>30.43%</td>
<td>5.7 ± 0.19</td>
<td>-1.12%</td>
<td>3.69 ± 0.19</td>
<td>-17.38%</td>
</tr>
</tbody>
</table>

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; Elzawahry 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.
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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$_2^-$ radical to H$_2$O$_2$ (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 $\gamma$-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 $\gamma$-irradiation showed restored to normal levels when compared to the corresponding values in DOX and/or $\gamma$-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 $\gamma$-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 𝑅 𝜸-irradiation when compared with the corresponding value of DOX and/or 𝑅 𝜸-irradiation. This reduction suggests that the used extract might be considered as a promising beneficial therapeutic agent against hepatotoxicity induced by DOX and/or 𝑅 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 𝜈-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 𝜈-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 𝜈-irradiation restored to normal levels when compared with the corresponding values of DOX and/or 𝜈-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.

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