1. INTRODUCTION

The liver is a crucial organ, and because of its strategic position, blood supply, and important function in metabolism, it is susceptible to drugs and chemicals to which we are constantly exposed (Gu and Manautou, 2012). Furthermore, the detoxification process in the liver eliminates the effects of xenobiotics with the aid of various microsomal enzymes. Hepatocytes have mitochondria more than other cells because they need more ATP to perform a variety of functions, resulting in higher ROS output within hepatocytes. As a result, hepatocytes are more vulnerable to oxidative stress-mediated toxic injuries. (Cichoz-Lach and Michalak, 2014, Dwivedi and Jena, 2018).

Cyclophosphamide (CP) is an immunosuppressant drug, and it is commonly used also to treat a number of cancers. (Fraiser et al., 1991, Khan and Jena, 2014, Patwa et al., 2020). However, due to significant adverse effects and toxicities in major organs such as the bladder, reproductive system, and liver, CP's clinical use is restricted (Patwa et al., 2020). Hepatic microsomal P450 oxidases metabolize CP in the liver, producing two main metabolites: phosphoramidase mustard and acrolein (Ramirez et al., 2019).

To control the CP-related adverse toxic effects, several methods have been used recently, including the use of Mesna (antioxidant) or alternative CP analogues, as well as a low dose of CP combined with another anticancer drug (Fisusi and Akala, 2019). These techniques, however, are insufficient and unsuitable for a wide variety of applications (Basu et al., 2014). As a result, an efficient and sufficient chemoprotective agent is urgently needed to minimize the toxic effects of CP while also increasing its therapeutic uses.

Many biological processes, including enzymatic reactions, require selenium as a trace element. Due to its protective potential against the reactive oxygen species (ROS), Selenium plays an important function in lowering chronic disease risks such as disorder of cancer neurodegenerative and hepatotoxicity (Teodor et al., 2011, Khan et al., 2012). At present, Se nanoparticles (SeNPs) are of great importance in medicine field because of their promising characteristics and excellent bioactivities, wherever they showed substantial impact as antitumor, toxic-free and biocompatible operators in comparison to the other selenium forms such as selenite (SeO3-2) and selenate (SeO4-2) compounds (Menon et al., 2018).
Therefore, the present research was designed to understand the ameliorative characteristics of SeNPs on hepatotoxicity triggered experimentally in albino rats.

2. MATERIAL AND METHODS

2.1. Experimental Animals
The experimental design of this study used fifty white male albino rats that were (10-12 weeks old) and weighed (140–160 g). Rats were collected from the laboratory animal’s research center, Mohshothor, Binha University’s faculty of veterinary. Rats were housed in standard light and temperature conditions and given free access to a standard pellet diet containing 21% protein, as well as tap water. Prior to the start of the experiment, the rats were given ten days to adjust.

2.2. Chemicals
Chemicals of analytical grade were obtained from trusted commercial suppliers. In the recent work chemicals used were:

2.2.1. Cyclophosphamide
Commercially available CP tablets (Endoxan® 50mg, Baxter Oncology GmbH).

Preparation of Cyclophosphamide (CP): The accurate doses of the drugs were dissolved in saline solution daily and shortly before administration at a dose of (5 mg/kg body weight) daily.

2.2.2. Sodium selenite from Sigma-aldrich, Egypt

Synthesis of nano-selenium
One ml of 25 mM sodium selenite (Sigma-Aldrich, Egypt) was combined with four ml of 25 mM glutathione containing either two mg or twenty mg of bovine serum albumin. To produce red elemental selenium and oxidized glutathione, the pH of the mixture was changed to 7.2 with 1.0 M sodium hydroxide. To isolate oxidized glutathione from selenium nanoparticles, the red solution was dialyzed for 96 hours at 4°C against double distilled water, which was replaced every 24 hours. The final solution, which included selenium nanoparticles and bovine serum albumin, was kept refrigerated at 4°C, in which, the selenium nanoparticle solution made with a low concentration of bovine serum albumin was stable for months, whereas the selenium nanoparticle solution made with a high concentration of bovine serum albumin was stable for years. (Abd-Allah and Hashem, 2015).

Preparation of nano-selenium
Nano-Se was synthesized and dissolved in saline (0.9 % NaCl) every day, just before being taken orally at a dose of (2mg/kg body weight) orally three times per week.

2.3. Experimental Design
Rats of the experiment randomly divided into five groups, 10 rats of each as follow:

2.3.1. Group (1): Negative control;
Rats were fed a normal diet without any medication for the duration of the work.

2.3.2. Group (2): Cyclophosphamide group "Positive group": Rats received CP orally at a dose of (5 mg/kg body weight) daily via gavage for 8 weeks (Gad El-Karim and El-Amravi, 2019).

2.3.3. Group (3): protective group" Nanoselenium group:
Rats received SeNPs (2 mg/kg body weight) orally three times per week via gavage during entire experimental period (Bhattacharjee, et al., 2014).

2.3.4. Group (4): Treated group "Cyclophosphamide + Nanoselenium group" Firstly rats received CP orally at a dose of (5 mg/kg body weight/8 weeks) daily via gavage, followed by receiving SeNPs (2mg/kg body weight/4 weeks) orally three times per week via gavage.

2.3.5. Group (5): treated group "Nanoselenium group + Cyclophosphamide" Firstly rats received SeNPs 2mg/kg body weight/4 weeks) orally three times per week via gavage as protector then, followed by orally at a dose of (5 mg/kg body weight/8 weeks) daily via gavage, with continuous administration with SeNPs.

2.4. Sampling
Rats in each group were fasted overnight and then euthanized after 12 weeks. At the end of the experiment, blood samples and liver tissue specimens were taken from all rat groups.

2.4.1. Blood samples:
Blood sample were collected from retro-orbital plexus of eye. Letting blood to clot, then, it was centrifuged at 3,000 rpm for 15 minutes. Sera were aspirated by automated pipette in Eppendorf. and kept at -20°C in a deep freezer until biochemical parameters were determined.

2.4.2. Tissue sample:
The liver was rapidly removed, washed with ice-cold salt, snapped in fluid nitrogen directly and held at -80°C.
One gramme of liver tissue was cut and minced into small parts, then homogenized with a glass homogenizer in 9 volumes of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10% homogenates, then centrifuged for 15 minutes at 6000 r.p.m. at 4°C. The antioxidant activity of the supernatant was measured directly.

2.5. Analysis

2.5.1. Biochemical analysis:
Biochemical parameters were estimated to evaluate liver enzymes activity as following: alanine aminotransferase (ALT) according to Fischbach, et al., (1992), aspartate amino transferase (AST) according to Fischbach, et al., (1992) and Alkaline Phosphatase (ALP) according to Z.Klin, (1970) by using Human kits for diagnosis (Germany). Also, albumin level was determined according to Gendler (1984) by using diagnostic kit supplied by (Diamond, Egypt).

2.5.2. Antioxidant estimation:
Furthermore, the supernatant of hepatic tissue homogenate (10%) was used for estimation of catalase (CAT) activity according to Fossati.et al., (1980), Superoxide dismutase (SOD) activity according to Nishikimi et al., (1972) and Glutathione peroxidase (GPx) activity according to (Paglia and Valentine et al.,1967) by using Biodiagnostic kit (Cairo, Egypt).

2.5.3. Cytokines estimation:
IL6 was measured by using Rat IL-6 Immunoassay, qantikine Elisa kit, (USA), Rat IL-1 beta ELISA Kit, Ray Biotech (USA) according to (Hirano, T. 1998) and (Auron et al., 1984) respectively.

2.6. Statistical analysis
SPSS software (version 19) was used to evaluate all gathered data. Differences between group means was identified by using (ANOVA), one-way analysis of variance, followed by the least significant difference (LSD) test. The minimum level of significance P-values were considered to be < 0.05.

3. RESULTS

3.1 Biochemical results
Administration of Cyclophosphamide to normal rats exhibited a significant increase in serum ALT, AST and Alp activity after 2 months of administration period when compared with negative control group. This elevation was reduced in SeNPs treated group when compared with untreated hepatotoxic group. However, Serum Albumin showed significant decrease in CP administrated group when compared to negative control, this reduction improved significantly in protected and treated SeNPs groups, data shown in table (1).

3.2 Antioxidants results
The results presented in our study revealed that, CP administration resulted in significant decreased liver SOD, catalase and GPx activity when compared with the control rats. SeNPs administration as a treatment or as a protection lead to significantly increased liver SOD and Catalase and GPx activity when compared to positive control lead to increased liver SOD, Catalase and GPx activity when compared with the control and untreated hepatotoxic groups. Serum Albumin showed significant decrease in CP administrated group when compared to negative control, this reduction improved significantly in protected and treated SeNPs groups, data shown in table (1).

3.3 Cytokines results
Increased inflammation of liver in CP administrated group was reported by increased levels of IL-1β and IL-6 compared to negative control. However, the amount of these inflammatory parameters in protected and treated with SeNPs groups decreased considerably when compared to CP treated group, such data shown in table (2).

4. DISCUSSION
Nanotechnology, the design and manipulation of materials at the atomic scale, has the potential to deliver considerable benefits to society. The novel properties that emerge as materials reach the nanoscale open the door to innovations in energy, manufacturing, and medical treatment. At the same time, these novel properties may pose new risks to workers, consumers, the public, and the environment. The limited data now available demonstrate the potential for some nano-materials to be persistent and mobile in the environment and in living organisms; to cross multiple physiologic barriers (including lung-blood, blood-brain, and placental barriers, and cell membranes) (Abd-Allah and Hashem, 2015). Selenium is an essential dietary trace element, which has an antioxidant role in the protection of the cell from oxidative damage. The most important metabolic roles of selenium in mammalian cell are due to its function in the active site of many antioxidant enzymes, e.g., thioredoxin reductase, glutathione (GPx) and GR enzyme in the active site of many antioxidant enzymes, e.g., thioredoxin reductase, glutathione (GPx) and GR.

Table 1 Effect of NanoSelenium administration on serum ALT, AST, ALP activity (U/L) and Albumin concentration (g/dl) in normal and hepatotoxicity groups of male albino rats, in comparison with control and hepatotoxicity untreated groups.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>ALT activity (U/L)</th>
<th>AST activity (U/L)</th>
<th>ALP activity (U/L)</th>
<th>Albumin Conc. (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>28.01 ± 1.33a</td>
<td>32.54 ± 1.89a</td>
<td>154.80 ± 6.77a</td>
<td>3.69 ± 0.15a</td>
</tr>
<tr>
<td>CP group</td>
<td>65.12 ± 1.93a</td>
<td>70.51 ± 3.55a</td>
<td>255.47 ± 7.32a</td>
<td>1.87 ± 0.09a</td>
</tr>
<tr>
<td>protective SeNPs</td>
<td>25.02 ± 1.52</td>
<td>29.23 ± 1.75d</td>
<td>149.83 ± 5.34d</td>
<td>3.44 ± 0.11b</td>
</tr>
<tr>
<td>Treated CP + SeNPs</td>
<td>44.04 ± 3.13b</td>
<td>51.46 ± 3.18b</td>
<td>182.15 ± 2.81b</td>
<td>2.80 ± 0.06b</td>
</tr>
<tr>
<td>Treated SeNPs + CP</td>
<td>35.03 ± 2.59c</td>
<td>37.83 ± 1.14c</td>
<td>163.80 ± 3.44c</td>
<td>3.05 ± 0.06c</td>
</tr>
</tbody>
</table>

Table 2 Effect of NanoSelenium administration on liver CAT, SOD and GPx activity (U/g. tissue) and proinflammatory markers (IL6-LI1 β) (pg/ml) in normal and hepatotoxicity groups of male albino rats, in comparison with control and hepatotoxicity untreated groups.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Antioxidants Activity</th>
<th>Cytokines level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAT (U/g.)</td>
<td>SOD (U/g.)</td>
</tr>
<tr>
<td>Control group</td>
<td>128.53 ± 10.07a</td>
<td>6.36 ± 0.21a</td>
</tr>
<tr>
<td>CP group</td>
<td>55.73 ± 4.39a</td>
<td>1.34 ± 0.24a</td>
</tr>
<tr>
<td>protective SeNPs</td>
<td>134.30 ± 7.90a</td>
<td>6.67 ± 0.18a</td>
</tr>
<tr>
<td>Treated CP + SeNPs</td>
<td>101.53 ± 5.89a</td>
<td>4.35 ± 0.40a</td>
</tr>
<tr>
<td>Treated SeNPs + CP</td>
<td>103.53 ± 6.92a</td>
<td>5.28 ± 0.34a</td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E), S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).
In this study we have shown that CP usage on rats had resulted in a significant increase in serum activity ALT, AST and ALP which indicating hepatocellular injury. The release of these enzymes from the liver cytoplasm in blood circulation could theoretically be the cause of this increase. However, the Nano-Se treatment has returned ALT, AST and ALP to normal patterns. Pre-treatment with Nano-Se has been more successful than post-treatment group with hepatocellular injury. Our results were in accordance with Bhattacharjee et al., (2014) who documented that treatment and protection with Nano Se reduced the hepatic damage and toxicity extent that affect directly liver enzymes. Moreover, Abdou and Sayed, (2019) who documented that the administration of the SeNPs had the ability to modulate the elevated levels of liver enzymes to be almost identical to negative control which may be attributable to the preservation of integrity or hepatocyte regeneration of damaged hepatocytes. The first line of defense against the reactive intermediates within the body can be considered CAT and SOD antioxidants. SOD is the first responsible for scavenging the superoxide radicals first responsible is SOD while CAT essential function is to neutralize the radical of hydrogen peroxide (Abdou and Sayed, 2019). Oxidative damage to DNA has been confirmed to be caused by cyclophosphamide's hydroperoxide by the production of H$_2$O$_2$ (Murata et al., 2004). The significant reduction of hepatic antioxidants (SOD and CAT) in a recent study can reveal their use in combating the pro-oxidants produced in CP metabolism and serve as a marker of tissue degeneration and injury (Ali et al., 2020). A single selenocysteine (Sec) residue is needed for enzyme activity in selenium-containing glutathione peroxidase (GPx) (Talas et al., 2010). GPx uses GSH to catalyse the reduction of hydroperoxidase, shielding mammalian cells from oxidative injury. Glutathione metabolism is, in fact, one of the most important antioxidative defence mechanisms (Bhattacharjee et al., 2014). The inability of the liver to produce the antioxidant enzyme GPx can explain the lower activity of this enzyme in our CP treated community. Treatment with SeNPs stopped GPx activity from being depleted in the liver, shielding the cell membrane from oxidative harm.

Results also showed that CP increased inflammatory damage, as evidenced by increased serum levels of pro-inflammatory cytokines IL-1β and IL-6. Toxic materials induce inflammation by stimulating macrophages and inducing the release of pro-inflammatory cytokines (Kang et al., 2012). Abdou et al., (2019) stated that IL-6 is usually produced due to inflammatory response induction and progression. As inflammatory reactions are mediated with IL-1 β through neutrophil activation (Segel et al., 2011). These findings are in consistency with Gangemi et al., (2016) and Ali et al., (2018), who conclude that the activation of redox-sensitive transcription factors that regulate the gene expression of pro-inflammatory mediators and antioxidants is linked to oxidative stress in the pathogenesis of inflammation.

Our findings showed that administering SeNPs reduced elevated liver enzymes and inflammatory markers to levels that were virtually identical to the control group; these findings may be due to the maintenance of hepatocytes or the regeneration of damaged hepatocytes (Patrick-Iwuanyanwu, et al., 2007). In addition, when compared to the Cp group, treatment with SeNPs increased antioxidant enzyme activity (CAT, SOD, and GPx) in liver tissue. These results support the restorative effects of SeNPs on liver tissue, which could be due to SeNPs' ability to reduce Cp-induced oxidative stress by inhibiting free radical chain reactions by reducing free radical output. Other researchers have confirmed SeNPs' antioxidative activity (Khalaf, et al., 2018, Zachara, 2015). Many studies have shown that nano Se has important protective effects against oxidative stress, DNA damage, and apoptosis, which are linked to Selenium's important function in improving antioxidative defense mechanisms and free radical scavenging ability within the cell (Fahmy et al., 2016).

5. Conclusion

Based on our results, we can conclude that Nano-Se is a promising Se formulation for the prevention of CP-induced hepatic injury. This research could pave the way for new ways to use Nano-Se as a hepatoprotective in the field of medicine.

6. REFERENCES

Abdel Magid et al. (2021) BVMJ 40 (2) : 100-104

oxidative stress in rats. Arch Toxicol., 76:269-76.


