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Disturbances in Thyroid Hormone Metabolism in Rats Exposed to γ- Radiation and Carbon Tetrachloride: The Role of N- acetyl cysteine and Sodium Selenite

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THIS work aims at detecting the disturbances in thyroid hormone metabolism in male rats exposed to liver injury by γ- radiation and/or carbon tetrachloride (CCl_4), in addition to investigating the modulatory role of N-acetyl cysteine (NAC) and sodium selenite (Se) supplementation. Subcutaneous injection of CCl_4 twice a week for 8 weeks and/or whole-body irradiation (6 Gy, single dose) one day after the last dose of CCl_4 induced hepatotoxicity as manifested by elevation of serum alanine aminotransferase and gamma-glutamyl transferase and oxidative stress in liver tissue. Moreover, significant increases were recorded in pro-inflammatory markers: nuclear factor kappa B and interleukin- 1beta levels. This was also accompanied by a significant decrease in the activity of type 1 iodothyronine deiodinase in the hepatic tissue and triiodothyronine and thyroxin levels in serum, while thyroid-stimulating hormone showed a non-significant increase. Histological examination of liver sections showed marked alterations. However, oral supplementation of NAC and Se for 2 weeks after exposure to CCl_4 and/or γ- radiation attenuated the changes induced by these toxins. In conclusion, supplementation of NAC & Se may be useful to attenuate thyroid hormone metabolic disturbances associated with liver injury induced by chemical and physical environmental toxins.

Keywords: γ-radiation, CCl_4, Liver injury, N-acetyl cysteine, Sodium selenite, Thyroid hormones.

Introduction

The liver is an important organ with various functions, including detoxification, glucose homeostasis, protein synthesis, and metabolism. Because the liver is involved in the metabolism of many hormones, liver injury may be associated with endocrine disturbances (Kumar et al., 2016). In respect to thyroid hormones, the liver plays an important role in their metabolism, as it is a vital organ in the peripheral conversion of prohormone, thyroxin (T_4) to triiodothyronine (T_3), the more active form, by the action of type-1 5'-iodothyronine deiodinase (5'-DI).

It is well known that oxidative stress can alter synthesis, activity, and metabolism of hormones. Previous studies demonstrated that exposure to physical (ionizing radiation) or chemical (carbon tetrachloride) environmental toxins induces an inhibition in the hepatic antioxidant enzymes associated with an increase in lipid peroxidation and a reduction in T_3 and T_4 levels in the circulation (Abdel Aziz, 2013; Ebrahim, 2020). Moreover, the cytokines elevated under the effect of oxidative stress disturb the activity of the three types of iodothyronine deiodinases. The two types 1 and 2 (5'-D1and 5'-D2) catalyze the activation of T_4 into T_3 through outer- ring deiodination, however, the type 3 (D3) catalyzes

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the inactivation of T_4 and T_3 through inner- ring deiodination. It was reported that IL-6 reduces the activities of D1 and D2 and increases the activity of D3 (Wajner et al., 2015).

Ionizing radiation is widely used in treating cancer and has vast applications in medicine and industries. Its harmful effects in different biological systems are due to the generation of free radicals. All reactive oxygen species (ROS) can react with critical biomolecules in the cells, such as DNA, membrane lipids and protein and other molecules, resulting in cellular damage (Xiao et al., 2014).

CCl_4 is one of the environmental toxins used in various industries. Its toxicity to human or experimental animals is largely due to its metabolic activation by cytochrome P450 2E1and the formation of the trichloromethyl radicals. Due to its high content of cytochrome P450, the liver is the major target of CCl_4 toxicity (Xu et al., 2017).

The deleterious effect of oxidative stress induced by exposure to different environmental toxins points to the necessity of developing multifunctional antioxidants. Therefore, a great deal of effort is being conducted to search for free radical scavenging agents that can counteract excess ROS and improve endogenous antioxidant system. Glutathione elevating agents, such as Taurine, alpha- lipoic acid and N-acetyl cysteine (NAC), are non-toxic (at certain levels), and were reported to protect tissues against radiation damage (El-Maraghi et al., 2018; Kim et al., 2019; Mercantepe et al., 2019). Recently, Abrigo et al. (2020) reported that NAC treatment inhibited all the mechanisms associated with myonuclear apoptosis in chronic liver disease induced muscle atrophy.

Selenium is an essential element involved in the regulation of cellular antioxidant capacity (Amuru et al., 2019) and thyroid hormone metabolism (Sobolev et al., 2018). Moreover, it was reported that selenium supplementation prevented Cd-induced hepatotoxicity through alleviation of endoplasmic reticulum stress and modulation of endoplasmic reticulum -resident selenoproteins (Zhang et al., 2020). Also, it improved tumor Necrosis Factor-alpha (TNF-α), interleukin-1beta (IL-1β), and vascular endothelial growth factor (VEGF) gene expression in infertile women who were selected for *in vitro* fertilization (Heidar et al., 2020). It was suggested that, in trauma patients, Se substitution may constitute an achievable adjuvant treatment strategy (Mareen et al., 2020).

This work is designed to investigate the disturbances in thyroid hormone metabolism in male rats exposed to liver injury induced by γ- radiation and/or CCl_4 and the role of NAC and sodium selenite (Se) in modulating these disturbances.

Materials and Methods

Chemicals

Carbon tetrachloride (98% purity) was purchased from El-Nasr Pharmaceutical Chemicals Co., Egypt. Sodium selenite (99% purity, CAS Number 10102-18-8) and NAC (99% purity, CAS Number 616-91-1) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used in this study were of highest purity and analytical grade.

Animals

Adult male Wistar rats aged 4–5 months and weighing 200–230 g were obtained from the animal house that belongs to the National Center for Radiation Research and Technology (NCRRT) Cairo, Egypt. Rats were fed a standard diet and provided water ad libitum. The animals were kept at 12 h light/dark cycle, at constant temperature (20 - 25°C) and humidity (50±5%). All experimental procedures were carried out according to the international guidelines of animal handling and care of the National Institute of Health (NIH publication No. 85 – 23, 1996) and the Ethics Committee of the NCCRT, Cairo, Egypt.

The liver injury was induced by the subcutaneous (S.c.) injection of CCl_4 (2 ml/Kg body weight) twice weekly for 2 weeks followed by subcutaneous injection of a reduced dose (1 ml/Kg body weight) twice weekly for 6 weeks as described by Constandinou et al. (2005), Ragab et al. (2019).

Radiation process

Whole-body gamma irradiation of the animals was performed using a Canadian Gamma Cell-40, (137Cs) at the NCCRT, Cairo, Egypt. The rats were exposed to a single dose of 6 Gy of γ-radiation with a dose rate of 0.398 Gy/ min, according to the Dosimetry and Protection Department in the NCRRT at the time of the experiment. The radiation dose was chosen to induce liver injury

without causing deaths in the rats (Abdel Aziz, 2013; Mansour et al., 2014).

Experimental design

The rats were divided into eight groups, 5 rats each. Group 1 (control) animals of this group were kept as control. Group 2 (NAC +Se) animals were orally supplemented with NAC dissolved in distilled water at a dose level of 200 mg/kg (according to Maheswari et al., 2014) and Se dissolved in distilled water at a dose level of 0.4 mg/kg (based on Said et al., 2012; Yang et al. 2019), daily for two weeks. Group 3 (Rad) rats were whole-body exposed to gamma radiation at a dose level of 6 Gy. Group 4 (CCl_4) rats of this group were injected with CCl_4 subcutaneously twice weekly for eight weeks to produce liver injury. Group 5 (CCl_4 + Rad) animals were injected with CCl_4 as group 4 and then exposed to 6 Gy gamma radiation (one day after the last dose of CCl_4). Group 6 (CCl_4 + NAC + Se) were injected with CCl_4 as group 4 and then received NAC and Se (one day after the last dose of CCl_4) as group 2. Group 7 (Rad + NAC + Se) rats were exposed to 6 Gy gamma radiation and received NAC and Se 5 minutes after irradiation as group 2. Group 8 (CCl_4 + Rad + NAC + Se) rats were injected with CCl_4 as group 4 then exposed to 6 Gy gamma radiation and received NAC and Se 5 minutes after irradiation as group 2.

Rats were sacrificed 24 h after the last dose of NAC and Se or 2 weeks after irradiation or the last dose of CCL_4. The blood samples were collected by heart puncture then centrifuged at 3000 rpm for 15 min, to separate serum. The serum samples were stored at -20°C until being analyzed. liver samples were immediately excised, rinsed with saline, dried by blotting with filter paper and then divided into two parts. One part was fixed in 10% formalin for histopathological examination and the other part was homogenized in phosphate-buffered saline (1g tissue: 10 ml PBS), centrifuged at 5000 rpm for 15 minutes at 4°C, then the supernatant was collected and preserved frozen at -20°C until using for biochemical analyses.

Biochemical analyses

Serum total T_3 and T_4 were measured using a radioimmunoassay kit (Cat. No: TKT3 1 and TKT4 1, respectively) and TSH in serum was measured using an immunoradiometric assay kit (Cat. No: IKTS1), Coat-A-Count (Siemens, Los Angeles, USA). IL-1β content in the hepatic tissue homogenate was estimated using Rat IL-1 beta ELISA Kit, Catalog No: MBS 825017, as described by the manufacturers. NF-κB level was determined in the hepatic tissue homogenate using (Rat Nuclear Factor KB ELISA Kit, Catalog No: MBS015549) kits following the manufacturer's guideline. Alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) activities were determined in the serum using spectrum diagnostic kits catalog No: 263001 and 246 002, respectively (Egyptian Company for Biotechnology; Cairo, Egypt) according to the manufacturer's directions. Activity of type 1 iodothyronine deiodinase (5'-D1) in the hepatic tissue homogenate was estimated using Rat ELISA Kit For Deiodinase Iodothyronine, Type I (DIO1), Catalog No: MBS2019735, according to the manufacturer's instructions.

Determination of Malondialdehyde level

The determination of lipid peroxidation was carried out according to Yoshioka et al. (1979). This method is based on the measurement of malondialdehyde (MDA) as the main end product of lipid peroxidation by the use of thiobarbituric acid (TBA). The reaction takes place in acid medium at temperature 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of this product is measured spectrophotometrically. In brief, 0.5 ml of liver homogenate was added to 2.5 ml of TCA (20%) and 1.0 ml of TBA (0.67%), and then the reaction mixture was shaken and heated for 30 min in a boiling water bath followed by rapid cooling. Then, 4.0 ml of n-butanol was added and shaken. The alcohol layer was separated by centrifugation at 3000 rpm for 10 min and transferred to the cuvette, and the absorbance of the resultant pink product was measured at 535 nm with using 1, 1, 3, 3-tetraethoxypropane as a standard.

Determination of reduced glutathione content

Reduced glutathione (GSH) content was performed according to Beutler et al. (1963). The method is based on measuring the intensity of yellow color developed after reduction of 5,5-Dithio-bis (2-nitrobenzoic acid) (DTNB), a disulfide chromogen, by sample GSH. The absorbance of the reduced chromogen is measured spectrophotometrically. This is directly proportional to GSH concentration. Briefly, 1.0 ml of liver homogenate was mixed with 3.0 ml of precipitating solution (1.67 g glacial metaphosphoric acid, 0.20 g disodium ethylenediaminetetraacetic acid, and 30 g sodium chloride dissolved in 100 ml water).

The mixture was centrifuged at 3000 rpm for 10 min, then 0.5 ml of the supernatant was mixed with 2.0 ml of phosphate buffer (0.2 M) and 0.25 ml of DTNB (0.04%) in 1% sodium citrate solution. The absorbance of the yellow color was measured at 412 nm within 4 min. GSH content was calculated based on a molar extinction coefficient of 13.6 Mcm−1 and a molecular weight of 307.

Determination of glutathione peroxidase activity

Estimation of glutathione peroxidase (GPx) activity was performed according to the methods of Gross et al. (1967). The method is based on the measurement of the amount of residual GSH left after exposure to GPx enzyme activity for a fixed time. Briefly, 0.5 ml of liver homogenate was mixed with 0.5 ml of reduced glutathione solution (0.008 M), 0.5 ml of sodium azide (0.03 M), 0.5 ml of disodium ethylenediaminetetraacetic acid (0.009 M) and 3 ml of distilled water and incubated at 37°C for 10 min. the reaction was initiated by the addition of 0.1 ml H_2O_2 (0.0018 M). The reaction was stopped by adding 3 ml of precipitating solution (glacial metaphosphoric acid (1.67%)). Then, it was centrifuged for 15 min at 4500 rpm. Residual GSH was determined by the same method used for the determination of reduced glutathione previously described by Beutler et al. (1963).

Histopathological examination

For the histopathological investigation, liver samples were fixed in formalin solution (10%) for 24 hours then washed in tap water, dehydrated in ethyl alcohol (ascending series), cleared in xylene and embedded in paraffin wax. Paraffin sections (4- 5 µm thickness) were sliced by microtome and stained with haematoxylin and eosin (H&E) reagent, according to Bancroft & Stevens (1996) and examined with a light microscope for assessment of histopathological changes. Slides were investigated by an independent histopathologist.

Statistical analysis

The SPSS computer program was used for statistical analysis of the results. Values were expressed as (mean± SE.). Statistical comparison between groups was conducted using one way ANOVA, followed by a post hoc, least significant difference. Differences were considered significant at P value < 0. 05.

Results

Biochemical analyses

The results presented in Tables 1-3 and Fig. 1 showed non-significant changes in all studied parameters upon supplementation of NAC+ Se to normal rats as compared to their normal control counterparts.

Irradiation of rats or CCl_4 administration or both treatments resulted in a significant elevation in the hepatic level of MDA together with a significant decrease in GSH level and GPx activity as compared to their values in the control group. However, NAC+ Se supplementation post-irradiation, CCl_4 or the dual treatments for 2 weeks induced a significant decrease in the level of MDA and a significant elevation in GSH content as compared with the corresponding values of irradiated, CCl_4 or both treatments (Fig. 1).

TABLE 1. Changes in serum alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and liver type 1 iodothyronine deiodinase (5'-DI) activities of adult male rats in different groups

Groups	ALT (U/L)	GGT (U/L)	5'-DI (ng/ mg protein)
Control	28.34± 0.67	4.02± 0.27	6.40± 0.37
NAC +Se	27.94 ± 1.06	4.06± 0.28	6.80± 0.33
Rad	38.08 ± 0.71[a]	4.94 ± 0.30 [a]	3.90± 0.27[a]
CCl_4	47.52± 0.76[a]	5.28± 0.28 [a]	2.60± 0.28[a]
CCl_4 +Rad	49.72± 0.73[a]	5.66 ± 0.24 [a]	2.26± 0.19[a]
Rad + NAC + Se	34.67± 0.86 [ab]	4.34 ± 0.22	5.10± 0.26 [ab]
CCl_4 + NAC + Se	38.48± 1.01[ab]	4.72± 0.30	4.34± 0.31[a b]
CCl_4 +Rad +NAC + Se	38.02 ± 1.29 [ab]	4.96 ± 0.21[a]	3.66± 0.33 [ab]

- Data are represented as means ± SE (n=5).

- a: Significantly different from the normal control group, b: Significantly different from the corresponding group that was not treated with NAC and Se. The mean difference is significant at the 0.05 level.

TABLE 2. Changes in liver nuclear factor kappa (NF-κB) and Interleukin 1 beta (IL-1β) levels of adult male rats in different groups

Groups	NF-κB (pg/ mg protein)	IL-1β (pg/ mg protein)
Control	39.96 ± 0.69	23.88 ± 0.72
NAC +Se	39.6 ±0.75	24.14 ± 0.77
Rad	56.84 ± 0.86[a]	35.90 ± 1.55 [a]
CCl_4	57.50 ± 0.92 [a]	39.82 ± 0.59 [a]
CCl_4 +Rad	60.20 ± 1.28 [a]	40.90 ± 1.10 [a]
Rad + NAC + Se	50.80 ± 2.60 [ab]	29.30±1.18 [ab]
CCl_4 + NAC + Se	50.40 ± 1.4 [ab]	34.40 ± 1.96 [ab]
CCl_4 +Rad + NAC + Se	50.20 ± 1.56 [ab]	30.60 ± 1.08 [ab]

- Data are represented as means ± SE (n=5).
- a: Significantly different from the normal control group, b: Significantly different from the corresponding group that was not treated with NAC and Se. The mean difference is significant at the 0.05 level.

TABLE 3. Changes in serum levels of triiodothyronine (T_3), thyroxin (T_4) and thyroid stimulating hormone (TSH) and T3/T4 of adult male rats in different groups

Groups	T_3(ng/dl)	T_4(µg/dl)	$T3/T_4$ e-3	TSH(µIU/ml)
Control	95.4 ± 2.29	5.14 ± 0.27	18.8 ± 0.49	0.79 ± 0.027
NAC +Se	96.0 ±2.32	4.92 ± 0. 17	19.6 ± 0.40	0.79 ± 0.024
Rad	69.4 ± 3.47[a]	4.50 ± 0.25[a]	15.6 ± 0.245[a]	0.84 ± 0.022
CCl_4	67.0 ± 3.19[a]	4.50 ± 0.19[a]	15.0 ± 0.32[a]	0.82 ± 0.022
CCl_4 +Rad	66.2 ± 2.08[a]	3.74 ± 0.24[a]	18.0 ± 0.63[a]	0.84 ± 0.024
Rad + NAC +Se	80.8 ± 3.51[ab]	4.62 ± 0.45	17.4 ± 0.40[ab]	0.79 ± 0.016
CCl_4 +NAC +Se	77.0 ± 2.98[ab]	4.66 ± 0.21	16.4 ± 0.245[ab]	0.81 ± 0.021
CCl_4 +Rad+NAC +Se	77.4 ± 2.89[ab]	4.44 ± 0.22[ab]	17.6 ± 0.245[a]	0.80 ± 0.021

- Data are represented as means ± SE (n=5).
- a: Significantly different from the normal control group, b: Significantly different from the corresponding group that was not treated with NAC and Se. The mean difference is significant at the 0.05 level.

The results in Table 1 revealed that exposure to ionizing radiation or CCl_4 administration or their combination resulted in a significant elevation in serum ALT & GGT activities and a significant decrease in liver 5'-D1 activity as compared to their values in control group. Oral supplementation with NAC + Se induced amelioration of the changes induced by exposure to radiation or CCl_4 as well as their combination in ALT and 5'-D1 activity. However, non-significant change was observed in GGT activity.

In Table 2 whole-body irradiation or CCl_4 administration or both treatments resulted in a significant elevation in the levels of pro-inflammatory markers, NF-κB and IL-1β as compared to their corresponding values of the control group. However, NAC + Se supplementation post-irradiation, CCl_4 or the dual treatments for 2 weeks induced a significant decrease in these levels although it still was higher than the normal control values.

The results presented in Table 3 revealed a non-significant increase in serum TSH level, however, significant decreases in T_3, T_4 and T_3/T_4 ratio were recorded after exposure to radiation or CCl_4 or their combination as compared to their corresponding values of the control group. NAC + Se supplementation post-irradiation, CCl_4 or the dual treatments for 2 weeks ameliorated the changes induced in T_3 and T_4 levels.

Histopathological examination

In the current study, The photomicrographs of the H&E staining liver sections demonstrated a well-developed hepatic architecture in control animals and those treated with NAC and Se (Fig. 2 A, B). On the

other hand, liver of rats exposed to radiation showed marked apoptosis with a mild vacuolar degeneration of hepatocytes, and mild intra-lobular inflammatory infiltrate (Fig, 2 C). Liver samples of rats exposed to CCl_4 alone or combined with radiation showed markedly dilated congested portal vein and marked vacuolar degeneration of hepatocytes in peri-venular area (Fig. 2 D, E). However, treatment with NAC and Se ameliorated the liver damage caused by irradiation and/ or CCL_4 administration (Fig. 2 F, G, H).

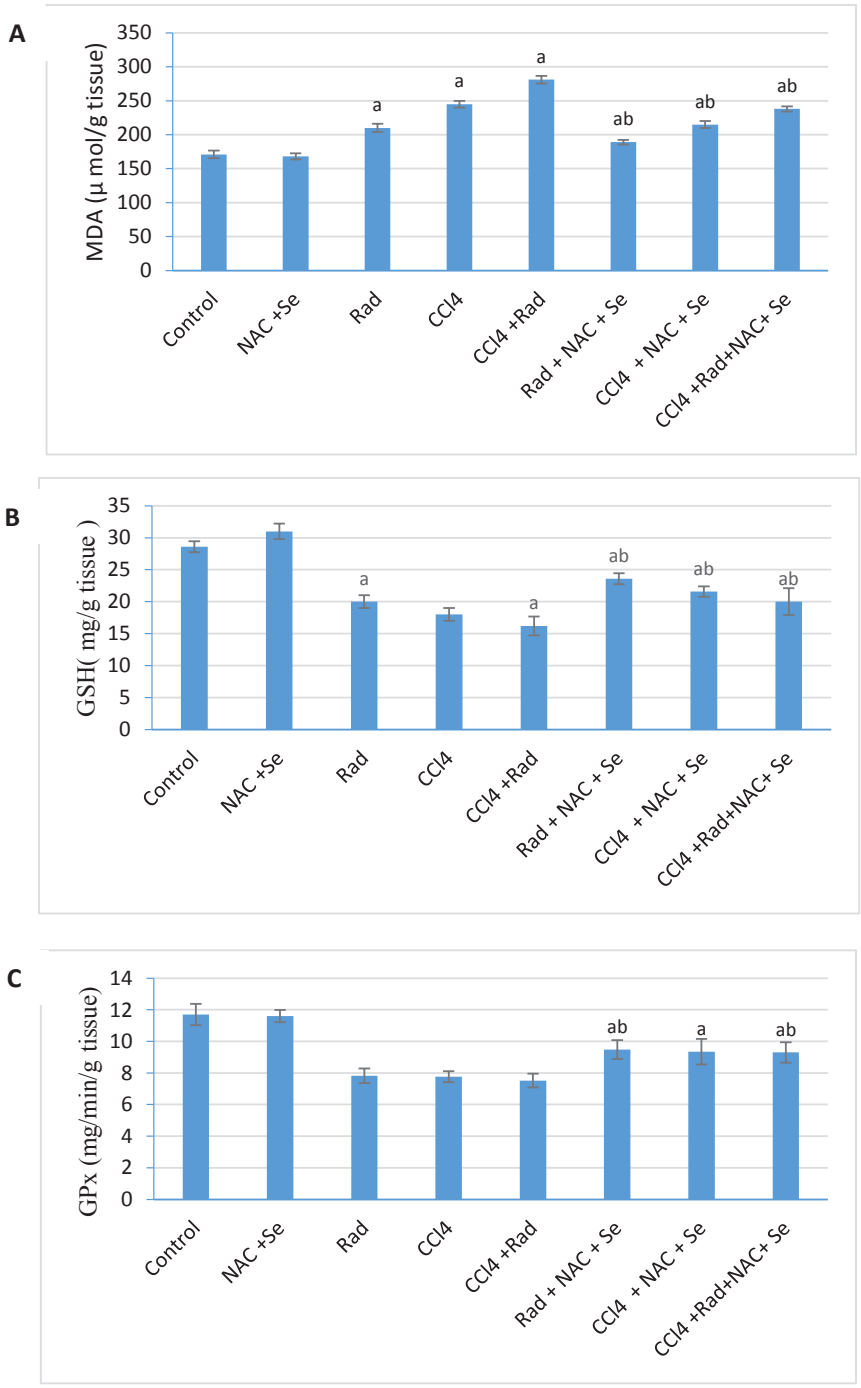

Fig. 1. Changes in liver malondialdehyde (MDA) (A) and glutathione (GSH) (B) levels and glutathione peroxidase (GPx) (C) activity of adult male albino rats in different groups [Data are represented as means ± SE (n=5). a: Significantly different from the control group, b: Significantly different from the corresponding group that was not treated with NAC and Se. The mean difference is significant at the 0.05 level]

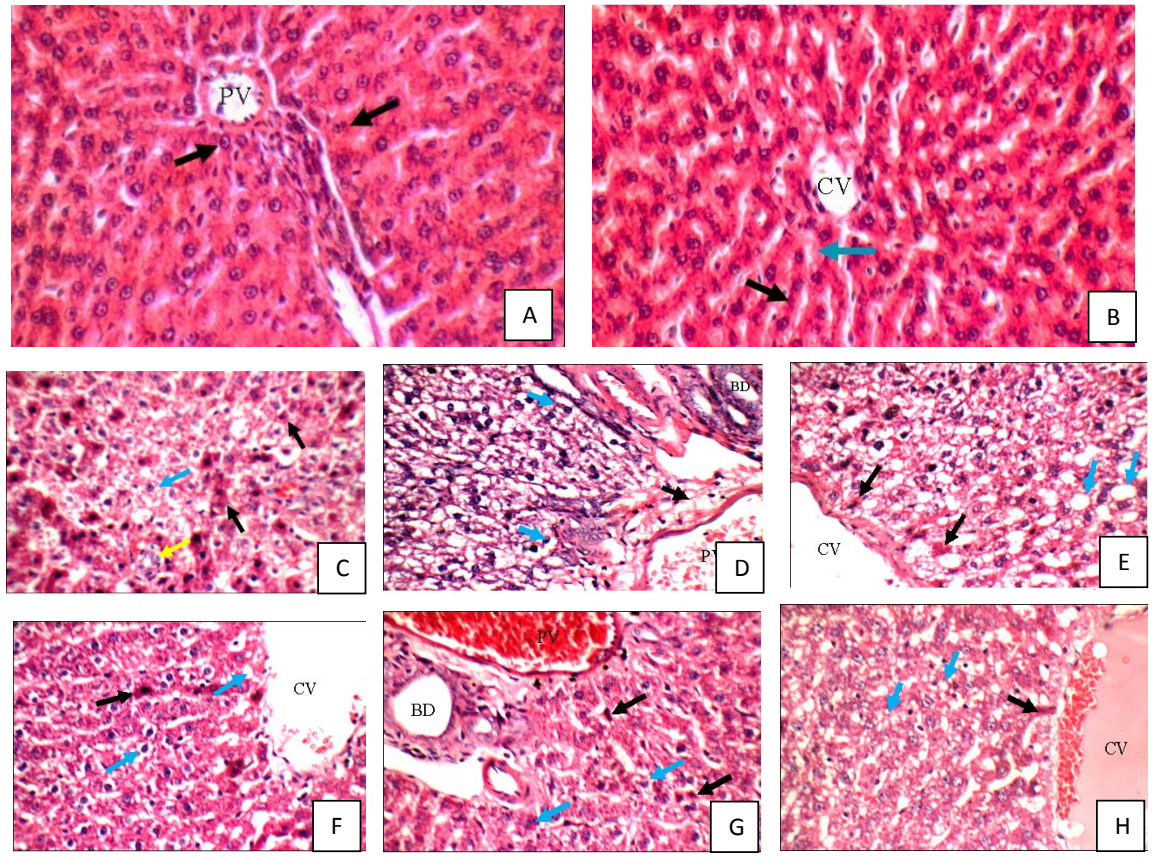

Fig. 2. Histological structure of rat liver in different groups [A, control: showing average portal tract with average portal vein (PV), and average hepatocytes in peri-portal area (black arrow). B, NAC+ Se: showing average central vein (CV) and average hepatocytes (black arrow) with average intervening blood sinusoids (blue arrow). C, Rad: showing marked apoptosis (black arrow) with mild vacuolar degeneration of hepatocytes (blue arrow), and mild intra-lobular inflammatory infiltrate (yellow arrow). D, CCL_4: showing mildly edematous portal tract (black arrow) with markedly dilated congested portal vein (PV), average bile ducts (BD), and marked vacuolar degeneration of hepatocytes in peri-portal area (blue arrow). E, CCL_4 +Rad: showing markedly dilated central vein (CV), scattered apoptosis (black arrow) and marked vacuolar degeneration of hepatocytes in peri-venular area (blue arrow). F, Rad + NAC + Se: showing mildly dilated congested central vein (CV), scattered apoptosis (black arrow) and mild vacuolar degeneration of hepatocytes in peri-venular area (blue arrow). G, CCL_4 + NAC+ Se: showing portal tract with mildly dilated congested portal vein (PV), scattered apoptosis (black arrow) with mild vacuolar degeneration of hepatocytes in peri-portal area (blue arrow). H, CCL_4 +Rad+ NAC+ Se: showing mildly dilated congested central vein (CV), scattered apoptosis (black arrow) and mild vacuolar degeneration of hepatocytes in peri-venular area (blue arrow). (H&E X 400)]

Discussion

Exposure of the mammalian body to high levels of environmental toxins induces complex cellular events, including oxidative stress, lipid peroxidation, inflammation and cytotoxicity that may undergo metabolic alteration leading to adverse outcomes in different organs of the body especially the liver. The pathogenesis of ionizing radiation or chemical toxin as CCl_4-induced liver injury involves the participation of free radicals and toxic metabolites that affect the physiological functions of the liver (Boll et al., 2001; Abdel-Fattah et al., 2013). Thyroid hormone metabolism is one of these functions.

As well known, antioxidant therapy has the potential to attenuate the injury induced by exposure to oxidative stress. NAC, which is a precursor of cysteine and glutathione, can suppress ROS production (Mercantepe et al., 2019). Also, sodium selenite, which can regulate the cellular antioxidant capacity (Amuru et al., 2019), has an important role in the metabolism of thyroid

hormones, where it is considered as an integral part of deiodinase enzymes responsible for activation and inactivation of thyroid hormones (Sobolev et al., 2018).

The results of the present study showed that whole-body irradiation at a dose level of 6 Gy and/or CCl_4 administration twice a week for 8 weeks induced a significant increase in MDA level associated with a significant decrease in GSH content and GPx activity in liver tissue. This is in addition to a significant increase in liver maker enzymes, ALT and GGT in serum. These results come in accordance with the previous studies which indicated that exposure to xenobiotic-induced lipid peroxidation generates free radicals that are likely to be quenched by cellular thiol compounds as reduced glutathione (GSH). Sulfhydryl group of GSH confers protection from cellular damage induced by oxidants and free radicals, which convert GSH to its oxidized form glutathione disulfide (GSSG). GSH acts as a substrate for GPx and GST, which remove toxic metabolites. So, the depletion of GSH under oxidative stress may further enhance lipid peroxidation (Vulimiri et al., 2011) and breakdown of polyunsaturated fatty acids associated with a decrease in the activities of endogenous antioxidants leading to cellular and subcellular membrane injury and subsequent release of the intracellular enzymes of hepatocytes into the bloodstream (Lee et al., 2019; Moradpour et al., 2020). The results may be indicative of the failure of the hepatic cells to respond to the oxidative stress induced by CCL_4 or radiation.

Therefore, it is beneficial to maintain GSH level and GPx activity for the prevention of CCl_4 or radiation-induced liver injury. The current study showed that NAC (Glutathione elevating agent) and sodium selenite supplementation inhibited the formation of MDA and increased the level of GSH and GPx activity in the liver and also decreased the activity of ALT in serum compared with that of the irradiated and/or CCl_4 treated groups. The results suggest that NAC and sodium selenite have the ability to increase the antioxidant capacity, quench free radicals and prevent cellular membranes from being damaged by free radicals. These results come in accordance with those of Mercantepe et al. (2019) who reported that NAC exhibited protective effects in rats against radiation-induced intestinal damage. Pei et al. (2018) demonstrated that NAC directly interacts with the free radicals via its thiol side – chain. In addition, it has an indirect antioxidant effect through increasing intracellular GSH level. Moreover, it was found that oral administration of NAC is more efficient than that of GSH due to its better absorption where it is rapidly and almost completely absorbed after oral administration; only 3% is excreted in the feces (Borgstrom et al., 1986). In addition, sodium selenite - the most commonly used form of Se supplements - possesses an antioxidant activity and it may have a therapeutic role in free radicals-mediated diseases (Merkord et al., 2017) and against the toxicity of different chemical and physical agents (Hanafi, 2007; Ahmadvand et al., 2014; Abdel Samie et al., 2017; Amuru et al., 2019). Since, Se is considered an essential part of different antioxidant enzymes, such as GPx and thioredoxin , it plays an important role in the antioxidant defense mechanisms in the body. An in vitro study showed that sodium selenite could regulate the antioxidant capacity, apoptosis rate, and expression of DNA methylation-related genes in pig splenic lymphocytes (Ren et al., 2019).

Inflammation- induced by exposure of animals to radiation or CCl_4 has an important role in hepatotoxicity. The inability of the liver to compensate for the imbalance of the redox system leads to the activation of the stress-sensitive signaling pathways or cascades as NF-κB. NF-κB is one of the central regulators in oxidative stress-induced inflammation through upregulation of several pro-inflammatory molecules as TNF-α, IL-1β, IL6, iNOS that culminate into hepatic injury (Alkhaklaf & Khalifa, 2018; Khayyal et al., 2019). The results of the present study indicated an increase in IL-1β associated with NF-κB in rats exposed to radiation and/or CCl_4. However, NAC and Se supplementation modulated these inflammatory markers in liver tissue. The anti-inflammatory effect of NAC takes place via the inhibition of NF-κB activity where NAC supplementation suppresses ubiquitination and degradation of NF-κB inhibitor; I-κB and thereby blocks NF-κB nuclear translocation and activation (Wu et al., 2014; Pei et al., 2018). It was suggested that ROS are important mediators of NF-κB activation and translocation to the nucleus in human conjunctival epithelial cells and NAC treatment significantly reduced nuclear NF-κB p65 protein expression and pro-inflammatory cytokines IL-6 and TNF-α (Park et al., 2015). In addition, Liu et al. (2017), suggested that

sodium selenite supplementation alleviated the inflammatory injury induced by Pb toxicity through inhibiting the NF-*κ*B signaling pathway and stimulating different selenoproteins in the chicken hearts. Also, it downregulated the gene expression of IL-1b and TNF-α in infertile women candidate for in vitro fertilization (Heidar et al., 2020). The histopathological examination of liver tissue supported the biochemical results and confirmed the ameliorative effect NAC and Se against ionizing radiation and / or CCl_4. These results are in agreement with the findings of Mercantepe et al. (2019) who observed that NAC manifested protective effect at the structural and molecular levels against gastrointestinal syndrome resulting from radiotherapy. Additionally, Se supplementation improved liver histopathological criteria against bisphenol A-induced hepatotocixity in rats (Abdel Samie et al., 2017).

The results showed that exposure of animals to radiation and/or CCl_4 induces a significant decrease in 5'-D1 activity associated with a significant decrease in circulating T_3 and T_4 levels and T_3/T_4 ratio. However, a non-significant increase in TSH level was observed. These results can be explained in view of the disturbance in the liver antioxidant system and lipid peroxidation and the consequent inflammatory response to liver damage which leads to a decrease in 5'- D1 activity and subsequently decrease the conversion of peripheral T_4 to T_3 (Punekar et al., 2018). Moreover, Wajner et al. (2015) reported that the increased levels of inflammatory cytokines lead to increases in free radical (as superoxide) production through the enzyme complex of the NADPH oxidase system and impairs D1and D2 (D2 is the enzyme responsible for conversion of T_4 to T_3, mainly expressed in brain, pituitary and brown adipose tissues) functions while inducing the expression of D3 (the enzyme responsible for inactivation of thyroid hormones). Because D2 is less sensitive to suppression by toxins, D1 is suppressed at levels that are lower than required to suppress the D2 in the pituitary (Holtorf, 2014). Thus, the non-significant changes in TSH could be explained in spite of the significant decrease in circulating T_3 and T_4. It could be suggested that the disturbances in circulating thyroid hormone levels after exposure to ionizing radiation and/ or CCl_4 administration may be secondary to the hepatotoxicity of these toxins. Moreover, impairment of thyroid function by these toxins (Khan et al., 2011; Rai et al., 2018) cannot be excluded. It was reported that exposure to ionizing radiation or chemical toxins interfere with the hypothalamus-pituitary-thyroid axis. It may prevent the binding of thyroid hormones to the thyroid hormone receptors that regulate gene transcription through interaction with thyroid hormone response elements in the promoter/ regulatory region of the specific genes, resulting in suppression of thyroid hormone receptors-mediated gene expression. This interference may be at the nuclear transcriptional level, particularly for T3-related genes or at the nongenomic level (Luksa-Lichtenthaler et al., 2000; Salam, 2013; Oliveira et al., 2019).

Supplementation of NAC and sodium selenite modulate the decrease in the hepatic 5'-D1, circulating T_3 and T_4 induced by exposure to radiation and/or CCl_4. Previous studies demonstrated that Se influences the expression and synthesis of selenoprotienase, 5'-D1 where it represents an integral part of this enzyme and is critical for protein function (Ruseva et al., 2013). However, Chakrabarti et al. (2016) reported that the role of the addition of selenium with L-thyroxin replacement in the treatment of hypothyroid patients remained inconclusive. In addition, Wajner et al. (2015) observed that Se supplementation did not restore D1 activity due to the depletion of endogenous thiol cofactor, the putative cofactor critical for the deiodinase function, under the effect of the oxidative stress and attributed that to the lack of Se effect on restoring the intracellular GSH levels. Therefore, the supplementation of NAC, the major cysteine donor to GSH and several thiol enzymes, together with Se in this work restored the intracellular GSH levels and prevented the inhibitory effect of oxidative stress on D1 activity and thyroid hormone levels. Moreover, NAC was shown to diminish type 3 iodothyronine deiodinase expression and activity in rats submitted to left anterior coronary artery occlusion (myocardial infarction), prevent the abnormalities in thyroid hormone metabolism and improve cardiac performance (Lehnen et al., 2017).

Conclusions

According to the results obtained in this study, oxidative damage and inflammation resulting from exposure to γ-radiation and/or CCl_4 exhibit a reduction in the hepatic 5'- D1 activity and

thyroid hormone levels in the blood. Oral supplementation of NAC together with Se modulated oxidative stress and inflammation in the liver and consequently modulated the disturbances in thyroid hormone metabolism.

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