Pathology - Research and Practice 240 (2022) 154186

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Short term deuterium depletion in drinking water reduced tumor induced oxidative stress in mice liver

Bertan Boran Bayrak [a,1], Gonul Yenidogan Kulak [b,2], Refiye Yanardag [a,3], Aysen Yarat [b,*,4]

[a] Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpaşa, 34320 Avcilar, Istanbul, Turkey

[b] Department of Basic Medical Sciences-Biochemistry, Faculty of Dentistry, Marmara University, Basibuyuk, 34854 Maltepe, Istanbul, Turkey

ARTICLE INFO

Keywords:
Ehrlich ascites tumor
Deuterium-depleted water
Liver
Oxidative stress
Antioxidants-oxidant
Enzyme

ABSTRACT

The aim of current work was able to show the oxidant effect of cancer cells found in any part of the body on the liver and to investigate the possible protective effect of deuterium-depleted water (DDW) on this oxidant effect by determining of some liver parameters. Ehrlich ascites tumor bearing BALB/c mice were used for this purpose. BALB/c mice were selected randomly and divided into four groups ($n = 5$ in each group) as control group, tumor group, control+DDW group, tumor+DDW group, fifteen days after tumor cell injection, liver tissue samples were taken for all groups. In the tumor group, liver lipid peroxidation, sialic acid and protein carbonyl levels, xanthine oxidase, myeloperoxidase, catalase, gamma-glutamyl transferase, sorbitol dehydrogenase, glutathione peroxidase and glutathione reductase activities, were significantly higher than those in the control group while glutathione levels and paraoxonase1, sodium potassium ATPase, glutathione-S-transferase, alanine transaminase and aspartate transaminase activities decreased significantly. Compared with the tumor group, the changes in all parameters except sialic acid, catalase, alanine transaminase and aspartate transaminase were reversed in the DDW given tumor groups, while sialic acid and catalase values continued to increase, and alanine transaminase and aspartate transaminase values continued to decrease. In conclusion, the consumption of DDW may be beneficial and protective against excessive oxidative stress in cancer complications.

1. Introduction

Cancer is an increasingly important health problem. It is a group of malignant diseases that occur as a result of uncontrolled proliferation and growth of cells that have been changed by some effects, both locally and at distant points. Cancer cells alter their metabolism to support their malignant properties [1]. Oxidative damage to macromolecules is thought to be an important etiologic factor in the development of several diseases including cancer. The reaction of free radicals (e.g., reactive oxygen species, ROS) with biomolecules generates molecular products which are generally considered to be more stable than ROS, therefore ROS levels are generally measured by determining stable metabolite concentrations of their target oxidation products [2,3].

Deuterium (D) is an isotope of hydrogen (H) with mass number two. Its chemical and physical properties are different from H, whose mass number is one. The possible role of D in organisms is still unknown. The effect of replacing H with D in biological systems has been reported. The D/H ratio is not constant in living organisms, it is variable [4].

The D concentration of natural water resources varies from region to region owing to geographical differentiations. The reason for the

Abbreviations: ALT, Alanine transaminase; ANOVA, Analysis of variance; AST, Aspartate transaminase; CAT, Catalase; DDW, Deuterium-depleted water; EAT, Ehrlich ascites tumor; GGT, Gamma-glutamyl transferase; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Reduced glutathione; GST, Glutathione-S-transferase; HBSS, Hanks' balanced salt solution; LPO, Lipid peroxidation; MDA, Malondialdehyde; MPO, Myeloperoxidase; Na^{+}/K^{+}-ATPase, Sodium/potassium adenosine triphosphatase; PC, Protein carbonyl; PON1, Paraoxonase1; ROS, Reactive oxygen species; SA, Sialic acid; SDH, Sorbitol dehydrogenase; SOD, Superoxide dismutase; TF, Tissue factor; XO, Xanthine oxidase.

* Corresponding author.

E-mail addresses: bertanb@iuc.edu.tr (B.B. Bayrak), gnlyndgn@gmail.com (G.Y. Kulak), yanardag@iuc.edu.tr (R. Yanardag), ayarat@marmara.edu.tr, ayaratster@gmail.com (A. Yarat).

[1] https://orcid.org/0000-0002-0700-5096
[2] https://orcid.org/0000-0003-2372-1276
[3] https://orcid.org/0000-0003-4185-4363
[4] https://orcid.org/0000-0002-8258-6118

https://doi.org/10.1016/j.prp.2022.154186
Received 12 July 2022; Received in revised form 7 September 2022; Accepted 20 October 2022
Available online 26 October 2022

difference in D concentrations in blood and urine is that D concentrations in drinking water are different [5,6]. In a pilot study in Turkey by Yarat et al. [7], D concentrations were determined in a hundred water samples obtained from 29 cities of 7 different regions of Turkey. D concentrations were found to be between 147 and 152 mgL^{-1}. The concentration of D in a healthy human blood is about 150 mgL^{-1}. This amount varies depending on age and gender. It has been reported that the D concentration in the plasma of cancer patients is 5–7 mgL^{-1} less than in the healthy ones [5]. Various clinical and experimental studies with deuterium-depleted water (DDW) have been conducted to date. According to the results of these studies, it has been suggested that DDW consumption regulates cellular respiration, increases cellular energy, has antioxidant, hypoglycemic, radioprotective, and antitumoral effects, has positive effects on mental functions, detoxification, and immune system [8–17].

Ehrlich ascites tumor (EAT) cells are preferred in experimental cancer research [18]. They are frequently used in the investigation of the effects of some drugs, especially drugs used in chemotherapy, on tumors [19]. There are studies examining the effect of DDW on oxidant damage in the liver [20–22]. EAT cells containing ascitic fluid were injected intraperitoneally (i.p) into each of the recipient mice. In fact, in this model, oxidant effect of cancer cells that may rapidly occur. We wanted to examine the short-term effect, namely the onset of cancer formation and thus to see the effect of DDW consumption at the very beginning.

The aim of current work was able to show the oxidant effect of cancer cells found in any part of the body on the liver and to investigate the possible protective effect of DDW (85 mg L^{-1}) on this oxidant effect by determining of some liver parameters.

2. Materials and methods

2.1. Chemicals

All chemicals were of reagent grade and used without further purification. They were supplied by Merck, Sigma-Aldrich and Fluka.

2.2. Animals and ethics

All the experimental procedures were approved by the Marmara University Animal Care and Use Ethics Committee (Protocol number: 64.2008.mar). Two and two half months old *Mus musculus* BALB/c type male mice between 18 and 25 g were obtained from Istanbul University Experimental Medicine Research Institute (DETAE).

BALB/c mice were selected randomly and divided into four groups ($n = 5$ in each group) as:

Group 1 (G1). : Control group given tap water for 15 days.

Group 2 (G2). : Tumor group given tap water 15 days.

Group 3 (G3). : Control group given DDW for 15 days.

Group 4 (G4). : Tumor group given DDW for 15 days after EAT injection.

In all groups, feeding was orally, water and feed administration were ad libitum carried out. The animals remained in their cages all the time. Nutrition and water (fresh fountain water) and DDW needs are met on a daily basis were stored under controlled laboratory conditions. In our experiment, DDW (Preventa) with a D concentration of 85 mgL^{-1} and tap water with a D concentration of 150 mgL^{-1} were used as drinking water. D concentration in water samples was determined in Ankara DSI Isotope Laboratory using a DUAL INLET type IRMS (Isotope Ratio Mass Spectrometer). For their feeding, the pellet type rat chow produced in Istanbul Cobancesme Feed Industry Factories was used. The metabolic energy it provides is 2650 $kcalkg^{-1}$. They were kept at the temperature of $20 \pm 2^{o}C$, with a 12 h light-dark cycle. DDW was given as drinking water to G3 and G4 groups.

2.3. EAT induction

EAT obtained from the donor animal from the Experimental Animal Breeding Unit of the Department of General Biology, Faculty of Science, Istanbul University, was used. With the help of a sterile syringe, the ascitic fluid containing EAT cells was taken from the peritoneal cavity of the donor mouse and suspended in Hanks' balanced salt solution (HBSS), some of this suspension is stained with trypan blue and counted in a hemocytometer. Viability was determined and the amount of ascitic fluid that should be administered to the recipient mice was determined. Acid (liquid) tumor formation was achieved by injecting 0.4 mL of ascitic fluid intraperitoneally (i.p) into each of the recipient mice in G2 and G4 groups, containing approximately 1.2×10^6 per mL tumor cells. Same volume of saline (0.9% NaCl) was injected to control groups. 15 days after tumor cell injection, liver tissue samples were taken for all groups.

2.4. Biochemical analysis

Liver tissue samples were dissected out, immediately washed in ice-cold physiologic saline solution, and then homogenized. These homogenates were centrifuged at 10.000 x *g* at $+ 4^{o}C$ for 10 min and then clear supernatants were used for the analysis. Reduced glutathione (GSH), lipid peroxidation (LPO, Malondialdehyde (MDA) equivalent), and sialic acid (SA) levels, and tissue factor (TF) activity were assessed by the methods described by Beutler [23], Ledwozyw et al., [24], Warren [25], and Ingram and Hills [26], respectively. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), and gamma-glutamyl transferase (GGT) were done according to the methods published previously by Aebi [27], Mylroie et al., [28], Paglia and Valentine [29], and modified by Wendel [30], Beutler [31], Habig and Jakoby [32], and Szasz [33], respectively. Moreover, myeloperoxidase (MPO) and xanthine oxidase (XO) activities were employed according to Wei and Frenkel [34] and Corte and Stirpe [35], respectively. Sodium/potassium ATPase (Na^+/K^+-ATPase), paraoxonase1 (PON1) activities, and protein carbonyl contents (PC) contents and sorbitol dehydrogenase (SDH) activities were determined by the methods of Ridderstap and Bonting [36], Furlong et al., [37], Levine et al., [38], and Barretto and Beutler [39], respectively. The total protein levels in supernatants were measured using procedure of Lowry et al., [40]. It was used to calculate for other parameters' values as per mg protein. Alanine transaminase (ALT) and aspartate transaminase (AST) were determined kinetically by Biolabo commercial kits (using Crony Jolly 100 spectrophotometer).

2.5. Statistical analysis

The biostatistical analysis of the study was carried out by Marmara University, Faculty of Communication, Department of Informatics. The NCSS statistical computer package was used. Variables are defined by mean and standard deviation (SD). In order to compare the means of measurement variables suitable for normal distribution, the "t" test was used in the comparison of two groups, and the paired t-test was used in the comparison of dependent samples of the same type of data. The Mann-Whitney U test was used to compare the non-normally distributed means. When comparing the mean of more than two groups and finding a difference "One-Way Analysis of Variance" (One-Way ANOVA) and Post-hoc Bonferroni, LSD tests or Kruskal Wallis, Post-hoc Dunn tests were used to interpret the differences between subgroups in variables. In interpretations, the limit of significance was taken as $P < 0.05$ was considered as significant.

3. Results

3.1. Effects of DDW on body weights, food and water consumption and cell counts of peritoneal fluid of EAT-induced animals

It was not seen a remarkable alteration between all groups for body weights. Daily food and water consumption were remarkably declined in the tumor group (G2) in comparison with the control group (G1). However, food consumption decreased, and water consumption increased significantly in tumor+DDW (G4) group compared to tumor group (G2) (Table 1).

Tumor cell count was performed in the peritoneal fluid. The number of cells injected as 1.2×10^6 per mL increased by 100 times in tumor groups at the end of the experiment. The number of viable cells in the peritoneal fluid in tumor groups (G2, G4) were $427 \times 10^8 \pm 0.72 \times 10^8$ and $462 \times 10^8 \pm 0.46 \times 10^8$, respectively. The administration of DDW did not cause a significant difference between the groups in terms of cell count ($P > 0.1$).

3.2. Effects of DDW on GSH, LPO, SA levels, and TF activity of EAT-induced liver tissues

Liver tissues GSH, LPO, SA levels, and TF activities of control and experimental groups of mice were significantly different ($P < 0.01$, Table 2). GSH decreased significantly in the tumor group (G2) in comparison with the control group (G1) ($P < 0.05$), while LPO and SA were significantly increased ($P < 0.001$), and the increase in TF activity was insignificant. Since the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity. In the control+DDW group (G3), GSH levels and TF activity increased significantly ($P < 0.05$) compared to control group (G1). In tumor+DDW group (G4), GSH values increased significantly compared to the tumor group (G2) ($P < 0.01$). LPO value was insignificantly decreased while TF and SA values were unremarkably increased in tumor+DDW group (G4) compared to the tumor group (G2) (Table 2).

3.3. Effects of DDW on CAT, SOD, GPx, GR, GST, and GGT activities of EAT-induced liver tissues

Liver tissues CAT, GPx, GR, GST, and GGT activities of control and experimental groups of mice were significantly different ($P < 0.01$ and $P < 0.001$, Table 3). There was no remarkable difference in SOD activities between the groups. Administration of DDW (G3) to the control group significantly increased CAT and GGT activities compared to the control group (G1) ($P < 0.001$), while SOD, GPx, GR, and GST activities insignificantly change. While CAT, GPx, GR, and GGT activities notably elevated in the tumor group (G2) in comparison with the control group (G1) ($P < 0.05$, $P < 0.001$, and $P < 0.001$), GST activity remarkably decreased ($P < 0.001$). Giving DDW to tumor group (G4) further reduced enzyme activities compared to the tumor group (G2) except CAT and SOD (Table 3).

Table 1
Body weights, daily food and water consumption of control and experimental groups of mice.

Groups	Body weight (g)*	Food (g/day)*	Water (mL/day)*
Control (G1)	28.35 ± 1.85	5.81 ± 0.0	6,09 ± 0.0
Tumor (G2)	28.38 ± 3.62	5.48 ± 0.0[c]	5,54 ± 0.0[c]
Control + DDW (G3)	26.75 ± 1.91	5,84 ± 0.0[c]	6.47 ± 0.0[c]
Tumor + DDW (G4)	28.88 ± 2.48	5,03 ± 0.0[f]	6.37 ± 0.0[f]
P_{ANOVA}	< 0.1	< 0.01	< 0.01

Abbreviations: EAT: Ehrlich Ascites Tumor; DDW: Deuterium-Depleted Water; G: Group; G1: Control group; G2: Tumor group; G3: Control+DDW group given DDW for 15 days; G4: Tumor+DDW group given DDW for 15 days after EAT injection.
[c] $P < 0.001$ versus Control group (G1); [f] $P < 0.001$ versus Tumor group (G2).
* Mean ± SD.

Table 2
Liver tissues GSH, LPO, SA levels, and TF activities of control and experimental groups of mice.

Groups	GSH (mg g protein^{-1})*	LPO (nmol MDA mg protein^{-1})*	TF (sec)*	SA (mg g protein^{-1})*
Control (G1)	1.43 ± 0.22	0.52 ± 0.09	83.13 ± 6.90	4.26 ± 0.72
Tumor (G2)	1.15 ± 0.10[a]	0.79 ± 0.07[c]	114.67 ± 36.50	8.83 ± 1.12[c]
Control + DDW (G3)	1.84 ± 0.36[a]	0.51 ± 0.05	74.50 ± 6.95[a]	4.12 ± 0.42
Tumor + DDW (G4)	1.42 ± 0.15[e]	0.72 ± 0.12	84.00 ± 20.31	9.84 ± 1.29
P_{ANOVA}	< 0.01	< 0.01	< 0.01	< 0.01

Abbreviations: EAT: Ehrlich Ascites Tumor; DDW: Deuterium-Depleted Water; G: Group; G: Group; G1: Control group; G2: Tumor group; G3: Control+DDW group given DDW for 15 days; G4: Tumor+DDW group given DDW for 15 days after EAT injection; GSH: Glutathione; MDA: Malondialdehyde; LPO: Lipid peroxidation; TF: Tissue factor activity; SA: Sialic acid
[a] $P < 0.05$; [c] $P < 0.001$ versus Control group (G1); [e] $P < 0.01$; versus Tumor group (G2).
* Mean ± SD.

Table 3
Liver tissues CAT, SOD, GPx, GR, GST, and GGT activities of control and experimental groups of mice.

Groups	CAT (U mg protein^{-1})*	SOD (U mg protein^{-1})*	GPx (U g protein^{-1})*	GR (U g protein^{-1})*	GST (U mg protein^{-1})*	GGT (U g protein^{-1})*
Control (G1)	10.11 ± 5.48	1.77 ± 0.094	230.50 ± 15.05	48.61 ± 0.99	1.76 ± 0.41	3.79 ± 0.81
Tumor (G2)	18.24 ± 7.13[a]	2.03 ± 0.31	306.10 ± 22.59[b]	59.09 ± 3.90[a]	0.49 ± 0.10[c]	11.53 ± 1.29[c]
Control + DDW (G3)	30.19 ± 8.67[c]	1.74 ± 0.17	228.10 ± 20.51	54.54 ± 3.17	1.47 ± 0.23	14.00 ± 0.73[c]
Tumor + DDW (G4)	20.62 ± 7.51	2.10 ± 0.17	254.40 ± 6.47	45.56 ± 3.46[e]	0.35 ± 0.16	4.24 ± 0.87[f]
P_{ANOVA}	< 0.01	> 0.05	< 0.001	< 0.001	< 0.01	< 0.001

Abbreviations: EAT: Ehrlich Ascites Tumor; DDW: Deuterium-Depleted Water; G: Group; G: Group; G1: Control group; G2: Tumor group; G3: Control+DDW group given DDW for 15 days; G4: Tumor+DDW group given DDW for 15 days after EAT injection; CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; GR: Glutathione reductase; GST: Glutathione-S-transferase; GGT: Gamma-glutamyl transferase
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ versus Control group (G1); [e] $P < 0.01$; [f] $P < 0.001$ versus Tumor group (G2).
* Mean ± SD.

3.4. Effects of DDW on ALT, AST, MPO, and XO activities of EAT-induced liver tissues

Liver tissues ALT, AST, MPO, and XO activities of all groups of mice were significantly different between groups ($P < 0.01$ and $P < 0.001$, Table 4). ALT and AST activities were notably decreased in the tumor group (G2) ($P < 0.01$ and $P < 0.001$) while MPO and XO activities remarkably elevated ($P < 0.05$) compared to the control group (G1). Administration of DDW to control group (G3) cause an insignificant change in ALT, AST, MPO, and XO activities. Enzyme activities were significantly decreased in tumor+DDW group (G4) ($P < 0.05$ and $P < 0.001$) compared to the tumor group (G2) (Table 4).

Table 4
Liver tissues ALT, AST, MPO, and XO activities of control and experimental groups of mice.

Groups	ALT (U mg protein^{-1})*	AST (U mg protein^{-1})*	MPO (mU g tissue^{-1})*	XO (U g protein^{-1})*
Control (G1)	1.23 ± 0.13	4.24 ± 1.14	58.70 ± 2.97	4.57 ± 0.29
Tumor (G2)	0.91 ± 0.19^{b}	0.57 ± 0.15^{c}	82.80 ± 7.21^{a}	6.47 ± 1.16^{a}
Control + DDW (G3)	1.25 ± 0.20	3.60 ± 0.75	43.53 ± 4.39	4.02 ± 0.45
Tumor + DDW (G4)	0.72 ± 0.17^{d}	0.36 ± 0.21^{d}	24.67 ± 10.68^{f}	4.29 ± 0.74^{d}
P_{ANOVA}	< 0.01	< 0.01	< 0.001	< 0.01

Abbreviations: EAT: Ehrlich Ascites Tumor; DDW: Deuterium-Depleted Water; G: Group; G: Group; G1: Control group; G2: Tumor group; G3: Control+DDW group given DDW for 15 days; G4: Tumor+DDW group given DDW for 15 days after EAT injection; ALT: Alanine transaminase; AST: Aspartate transaminase; MPO: Myeloperoxidase; GGT: Gamma-glutamyl transferase; XO: Xanthine oxidase
$^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$ versus Control group (G1); $^{d}P < 0.05$; $^{f}P < 0.001$ versus Tumor group (G2).
* Mean ± SD.

3.5. Effects of DDW on Na^{+}/K^{+}-ATPase, PON1 activities, PC levels, and SDH activity of EAT-induced liver tissues

Liver tissues Na^{+}/K^{+}-ATPase, PON1 activities, PC levels, and SDH activities of all groups of mice were significantly different ($P < 0.001$, Table 5). Na^{+}/K^{+}-ATPase and PON1 activities were markedly abated in the tumor group (G2) ($P < 0.001$) when compared with the control group (G1). Na^{+}/K^{+}-ATPase and PON1 activities were notably increased in tumor+DDW group (G4) ($P < 0.001$ and $P < 0.01$) compared to tumor group (G2). Giving DDW to the control group (G3) led to an increase in Na^{+}/K^{+}-ATPase activity ($P < 0.001$) and however, a decrease in PON1 activity ($P < 0.01$) compared to the control group (G1) (Table 5). PC levels and SDH activities increased significantly in the tumor group (G2) ($P < 0.001$) when compared to the control group (G1). PC levels and SDH activities decreased significantly with DDW administration to tumor group (G4) ($P < 0.001$). DDW administration to the control group (G3) did not significantly change the PC value and SDH activity (Table 5).

Table 5
Liver tissues Na^{+}/K^{+}-ATPase, PON1 activities, PC levels, and SDH activity of control and experimental groups of mice.

Groups	Na^{+}/K^{+}-ATPase [nmol $_{Pi}$ (mg protein.h)$^{-1}$]*	PON1 (U mg protein^{-1})*	PC (nmol mg protein^{-1})*	SDH (U g protein^{-1})*
Control (G1)	1.15 ± 0.07	9.92 ± 0.99	4.29 ± 0.33	3.84 ± 1.25
Tumor (G2)	0.37 ± 0.12^{c}	5.12 ± 0.39^{c}	7.85 ± 0.46^{c}	14.22 ± 2.45^{c}
Control + DDW (G3)	2.14 ± 0.22^{c}	7.67 ± 1.15^{b}	3.87 ± 0.47	3.01 ± 0.42
Tumor+ DDW (G4)	2.00 ± 0.16^{f}	7.62 ± 1.59^{e}	4.86 ± 0.58^{f}	8.06 ± 1.51^{f}
P_{ANOVA}	< 0.001	< 0.001	< 0.001	< 0.001

Abbreviations: EAT: Ehrlich Ascites Tumor; DDW: Deuterium-Depleted Water; G: Group; G: Group; G1: Control group; G2: Tumor group; G3: Control+DDW group given DDW for 15 days; G4: Tumor+DDW group given DDW for 15 days after EAT injection; Pi: Inorganic phosphate; Na^{+}/K^{+}-ATPase: Sodium/potassium ATPase; PON1: Paraoxonase1; PC: Protein carbonyl; SDH: Sorbitol dehydrogenase
$^{b}P < 0.01$; $^{c}P < 0.001$ versus Control group (G1); $^{e}P < 0.01$; $^{f}P < 0.001$ versus Tumor group (G2).
* Mean ± SD.

4. Discussion

Cancer is the most serious disease of the modern age we live in. While cancer-related deaths are in the first place in developed societies, it is increasing gradually in developing countries. Today, very serious efforts and large amounts of budgets are spent in the fight against cancer. However, cancer remains the first factor threatening health.

The reason why EAT cells are preferred in experimental cancer research is that they have a very high transplant rate and do not show regression. This model cannot be applied to experimental animals other than mice. Rejection is observed in experimental animals other than mice [41]. The origin of EAT cells comes from the spontaneous-onset mammary adenocarcinoma of the mouse [42]. The liquid form of this tumor growing in the peritoneum of mice was obtained [43]. Liquid EAT has been a tumor that is frequently used in the investigation of the effects of plant extracts as well as some drugs, especially drugs used in chemotherapy, on tumors [44–46]. The chemotherapy is a promising strategy for preventing the relapse of cancer patients being in remission but not for healthy populations.

There are very few studies in the literature investigating the effect of DDW consumption on liver. The consumption of DDW for 42 days gives rise to the decline in the D concentration in the plasma and tissues (liver and kidney) without causing any toxic effects. Moreover, it was put forward that isotope exchange reactions due to isotopic D/H composition of the tissues caused by altered DDW, thereby may influence both the cell cycle and proliferation [20]. In the other studies regarding the effects of DDW and some herbal essential oils (in combination) on experimental liver damage has been reported that their combination gives rise to synergistic protective activity, thereby restoring the alterations of the several oxidative stress and inflammatory parameters [21, 22].

In the present study, the effects of DDW on body weights, daily food and water consumption and some liver parameters such as indicators of tissue damage and antioxidant system were investigated in EAT-treated BALB/c mice. 15 days after the EAT injection, it was found that body weights were not significantly altered whereas daily food and water consumption were remarkably declined in the tumor group (G2) in comparison with the control group (G1). Food consumption decreased and water consumption increased significantly in tumor+DDW (G4) group compared to tumor group (G2). On the other hand, LPO, SA, and PC levels CAT, GPx, GR, GGT, MPO, XO, and SDH activities in the liver were notably elevated in the tumor group (G2) as compared with the control group (G1), while GSH levels and GST, ALT, AST, Na^{+}/K^{+}-ATPase, and PON1 activities were found to be decreased significantly.

Reduced GSH, one of the major intracellular components of antioxidant defense, is responsible for not only the neutralization of free radicals but also regulations of cellular survival by maintaining redox balance in a variety of tumors [47,48]. The increase in the amount of GSH in the tissue is an indication that the cells are protected from oxidative stress. Decreased GSH level may affect the development of the disease [49]. In our study, liver GSH was remarkably declined in the tumor group (G2) when compared with the control group (G1). DDW reversed the decrease in GSH level in tumor group (G4).

Liver tissue has antioxidants that prevent damage caused by excess oxygen metabolites. These antioxidants neutralize both peroxides and free radicals [50,51]. It is emphasized that antioxidant enzyme activities elevate so as to accommodate adaptive mechanisms in case of increased oxidant stress such as cancer [52]. In the case of cancer and several diseases (e.g., diabetes, myocardial infarction, ailments of respiratory, nervous, and urinary systems) in the stress and aging processes, remarkable alterations in antioxidant enzymes and an increase in LPO levels have been reported by many researchers [53–55]. LPO is one of the important causes of cell membrane damage and destruction. Polyunsaturated fatty acids react with free radicals and increased LPO results in accumulation of MDA which is mutagenic and possibly carcinogenic [56].

TF, a transmembrane glycoprotein, acts as a hemostatic envelope for tissues and blood vessels. When binding to plasma protein factor VII, TF initiates clotting, thus being released into the bloodstream and initiates clotting in damaged tissues [57]. In our study, insignificant alteration was found in TF activity between the control and tumor groups. However, while DDW application caused an increase in TF activity in the control group (G3), it caused a decrease in tumor group (G4). Considering the low TF activity is inversely associated with clotting time, in the current study DDW can be thought to prevent the risk of bleeding in the control group and prevent the risk of clotting in the tumor groups.

Studies have shown that tumor cells carry enzymes that synthesize different types of glycoproteins and that the synthesized substances contain a large amount of SA [58]. SA is also synthesized in the liver. Cell formation and destruction in tumor tissue is much higher than in normal tissue. The increase in SA in the cell membrane allows the cell adhesion to decrease and the tumor cell to invade easily. Thus, SA is effective in the development of near and distant metastases by invading the vessels of the tumor cell [59]. It is also argued that SA masks the antibody receptors on the tumor cell surface, causing these cells to escape from immune control and is directly responsible for the development of malignancy [60]. Parallel to these studies, in the present study, SA values were significantly increased in the liver of the tumor group (G2) compared to the control group (G1). Administration of DDW could not correct the significant increase in SA levels in tumor group (G4), and even caused more increase in them. The effect of DDW may not be related SA metabolism and should be investigated in detail.

The imbalance between pro-oxidant (ROS)-antioxidant enzymes/levels shifting in favor of ROS gives rise to an alteration in the activities of the antioxidant enzymes (e.g., CAT, SOD, GPx, GR, and GST). These are also the basic and first-line defenses against detrimental effects of ROS-mediated oxidative stress [61]. SOD dismutases superoxide anion radical to molecular oxygen and H_2O_2. The latter is oxidized to H_2O by either CAT or GPx. GPx decomposes H_2O_2 to H_2O by utilizing GSH co-substrate concomitant to oxidation to GSSG. Meanwhile, GSSG is, in turn, converted to GSH by the catalytic effect of the GR enzyme [62]. In the present study CAT, GPx, and GR activities increased in the tumor group (G2) compared to the control group (G1). However, SOD did not change significantly. DDW caused the increase in CAT activity, the decrease in GPx and GR activities in the tumor group (G4) compared to the tumor group (G2). Our findings were not in harmony with Rasooli et al., [63] who has been revealed that activities of GPx and GR enzymes remarkably elevated as a result of acetaminophen-mediated hepatotoxicity, but DDW administration gave rise to a dramatical restoration of these enzymes' activities. The discrepancy of effect of DDW may be associated with its prooxidant properties according to consumption time of DDW [64].

Stimulation and expression of GGT and GST, the enzymes of glutathione metabolism, can easily occur via exposure of the organism to several initiators and/or promoters (e.g., chemical carcinogens, hormones, and diet). It has been suggested that GGT expression is generally significantly increased in people with cancer and that GGT can be used as an indicator of cancer risk [65]. Stimulation of GGT and induction of GST have been shown to allow GSH depletion so as to the protection of hepatocytes against deleterious effects of exposure to xenobiotics. Thus, the combined effects of GGT and GST in a toxic environment could provide the enhanced proliferation observed in preneoplastic hepatocytes [66]. The present study showed that GGT activity increased, and GST activity decreased in the liver tissue of the tumor group (G2) compared to the control group (G1). The decrease in GST may be due to excessive increase in GGT activity. DDW reversed the increase in GGT activity in tumor group (G4). GST activity continued to decrease in tumor+DDW group (G4). The difference in the GGT activity between control group (G1) and control+DDW (G3) groups was significantly changed. The increase in the GGT activity in the group (G3) was as much as in the tumor group (G2). In the control group DDW may induce GSH formation by increasing GGT activity. However, the GST activity were not significantly different between control groups (G1 and G3).

The liver is one of the most important organs in the body with its functions of synthesis and detoxification [67]. In many diseases, liver tissue is directly or indirectly affected. ALT is a cytoplasmic enzyme found mostly in the liver. It is not found in other tissues or very less amount. Therefore, the elevation of this enzyme is considered as a specific finding of liver diseases. On the other hand, AST, which is both a cytoplasmic and mitochondrial enzyme, is also found in heart and skeletal muscles besides the liver. It is found and may increase in events involving the heart and skeletal muscle, as well as hepatocellular damage [68,69]. In the present study, liver ALT and AST enzyme activities were markedly declined in the tumor group (G2) in comparison with the control group (G1), contrary to expectation. Administration of DDW could not correct the significant decrease in ALT and AST activities in tumor group (G4), and even caused more decrease in them. However, in the control+DDW group (G3), ALT and AST values did not change compared to the control group (G1). This may be due to DDW triggering ROS generation by changing the mitochondrial membrane potential or by acting as a prooxidant [70]. Or the reason for the decrease in the activities of ALT and AST enzymes may be related to the adaptation process of hepatocytes to DDW or possibly due to the alteration of DDW-mediated transmembrane gradient [10].

MPO and XO were the enzymes that increased in the tumor group compared to the control group in the present study. It has been proposed that chronic inflammation is associated with the risk of malignancy [71]. MPO, one of the enzymes containing heme in its structure, catalyzes the generation of hypochlorous acid. Hypochlorous acid is a powerful oxidant with bactericidal activity. Therefore, MPO causes neutrophil cytotoxicity [72]. Another reason for the onset and progression of diseases such as cancer is the excessive increase of these oxidants and tissue damage due to the effect of MPO [73]. XO is an enzyme that has both dehydrogenase and oxidoreductase activities, which catalyzes the oxidation of hypoxanthine and xanthine to uric acid [74]. The highest levels of XO are found in the liver, though it has also been found in cardiac, pulmonary, and adipose tissue. In the organism, free radicals are continuously generated in normal cellular function. In addition to this, XO also contributes to the formation of free radicals such as superoxide anion and H_2O_2 [75]. In the present study, DDW consumption reversed the increase in MPO and XO activities in tumor group (G4). However, in the control+DDW group (G3), MPO and XO activities did not change compared to the control group (G1). The anticancer effect of DDW may because of its inhibiting cell proliferation by causing an imbalance between ROS production and neutralization in mitochondria. Thus, oxidative stress is induced in the cell [70].

Na^+/K^+-ATPase belongs to the enzyme class of translocases. It has numerous cellular functions. It has an essential role in maintaining the ionic and osmotic balance in the cell, and a receptor function in signal transductions [76]. Depending on the cell type or respective ligand, Na^+/K^+-ATPase stimulates the proliferation of healthy cells [77,78], or contrarily, inhibits the proliferation of tumor cells [79]. The changes in the activity and expression of Na^+/K^+- ATPase may be associated with the pathogenesis of many diseases [80]. Na^+/K^+-ATPase has been considered as a target for drugs, especially those with antitumor activities [81]. According to our findings, activities of Na^+/K^+- ATPase decreased significantly in the tumor group (G2) compared to control group (G1). The activity of this enzyme may be impaired owing to excessive oxidative stress that can give rise to necrotic abnormalities in the liver. The decrease in the activities of this enzyme may result from elevated LPO levels in the tumor groups, which can disrupt membrane integrity and oxidize the specific sulfhydryl residues present in its active binding pocket in response to aberrant oxidative stress [82]. In the present study, DDW reversed the decrease in Na^+/K^+-ATPase activity in tumor group (G4) by reduction of oxidative stress.

Paraoxonases (e.g., PON1, PON2, and PON3) are HDL-binding glycoproteins that act as cellular antioxidants [83]. Moreover, it has been reported to involve in the pathogenesis of various inflammatory diseases such as cardiovascular complications, diabetes, and cancer. PON1 has also hydrolytic activity depended on Ca^{2+} [83]. The decline in the activity of PON1 has been reported to be associated with cellular damage and tissue injury [84,85]. Our study reveals that the decrease of PON1 in liver tissue of tumor group (G2). That means the diminish in the antioxidant capacity. DDW reversed the decrease in PON1 activity in tumor group (G4). This elevation can be associated with the antioxidant property of DDW. However, DDW caused the decrease in control group (G3) compared to control group (G1).

Protein carbonylation can easily occur by directly or indirectly oxidative modification of carbonyl groups at the side chains of specific amino acids (e.g., L-arginine, L-lysine, L-proline, and L-threonine) in proteins as a result of oxidative stress, thus occurrence of PC in the polypeptide chain may be strongly related to oxidative tissue injury [86]. It has been shown that the elevations of both PC and LPO (measured in terms of MDA equivalent) levels can be the most useful biomarkers for determining oxidative stress status in various diseases including cancer and other malignancies [84,87]. In the present study, DDW consumption reversed this increase in LPO and PC values in tumor group (G4). However, in the control group (G3) given DDW, LPO and PC values did not change in comparison to the control group (G1). The ability of water with reduced D content may cause to increase the potential of the organism defense system. The nutritional correction of isotope D/H exchange by DDW in the organism may modify isotope composition and corrects imbalanced function of the body's defense systems [88].

SDH is the second enzyme of the polyol pathway. In addition to the glycolytic and pentose phosphate pathways, the polyol pathway has been suggested to be another means of tumor-related depletion of glucose due to the critical role of SDH. It has been put forward that in tumorigenesis and advanced cancer cells consume glucose much higher than normal cells, and the rest of the subsequent energy is obtained from the glycolytic pathway compared to oxidative phosphorylation of glucose [89]. The present study reveals that the increase of SDH activity in liver tissues of tumor group (G2). DDW consumption reversed the increases in SDH activity in tumor group (G4). However, in the control group (G3) given DDW, SDH activities did not change compared to the control group (G1). Consuming more glucose at a much higher rate than normal cells may of cause formation or ROS. DDW may prevent oxidative stress by decreasing ROS formations.

5. Conclusion

Reducing the D concentration from 150 mgL^{-1} to 85 mgL^{-1} in drinking water was very effective in decreasing oxidative stress. DDW consumption may be beneficial in cancer with excessive oxidative stress and may have a protective effect against the complications of cancer. Since the concentration of DDW and the duration of use are also important, more detailed studies are necessary to determine the useful dosage and application time, along with studies to illuminate the mechanism of action of DDW.

Funding

This study was financially supported by the Marmara University Research Fund [Grant number: SAG-C-YLP-270109-0003].

Ethics approval

All the experimental procedures were approved by the Marmara University Animal Care and Use Ethics Committee (Project number 64.2008.mar).

CRediT authorship contribution statement

Bertan Boran Bayrak, Gonul Yenidogan Kulak, Refiye Yanardag, Aysen Yarat: Conceptualization. **Bertan Boran Bayrak, Gonul Yenidogan Kulak, Refiye Yanardag, Aysen Yarat**: Methodology, Formal analysis, Investigation, Data curation. **Refiye Yanardag, Aysen Yarat**: Resources, Visualization, Supervision. **Aysen Yarat**: Project administration, Funding acquisition. **Bertan Boran Bayrak, Gonul Yenidogan Kulak, Refiye Yanardag, Aysen Yarat**: Writing – original draft. **Bertan Boran Bayrak, Gonul Yenidogan Kulak, Refiye Yanardag, Aysen Yarat**: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Prof. Dr. Seyhan Altun and Ayşe Topuz, from Istanbul University Faculty of Science Department of General Biology, for helping us to obtain the donor animal carrying Ehrlich Ascites Tumor and whose information we consulted about the application method. We would also like to thank Prof. Dr. Ümit Ş. Zeybek, one of the Faculty members of the Istanbul University Experimental Medicine Research Institute, Department of Molecular Medicine, for his help and support.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

References

[1] S.H. Hassanpour, M. Dehghani, Review of cancer from perspective of molecular, J. Cancer Res. Pract. 4 (2017) 127–129, https://doi.org/10.1016/j.jcrpr.2017.07.001.
[2] S. Arfin, N.K. Jha, S.K. Jha, K.K. Kesari, J. Ruokolainen, S. Roychoudhury, B. Rathi, D.M. Kumar, Oxidative stress in cancer cell metabolism, Antioxidants 10 (5) (2021) 642, https://doi.org/10.3390/antiox10050642.
[3] J.D. Hayes, A.T. Dinkova-Kostova, K.D. Tew, Oxidative stress in cancer, Cancer Cell 38 (2) (2020) 167–197, https://doi.org/10.1016/j.ccell.2020.06.001.
[4] G. Somlyai, G. Jancso, G. Jakli, K. Vass, B. Barna, V. Lakics, T.M. Gaal, Naturally occurring deuterium is essential for the normal growth rate of cells, FEBS Lett. 317 (1–2) (1993) 1–4, https://doi.org/10.1016/0014-5793(93)81479-J.
[5] P. Berdea, S. Cuna, M. Cazacu, M.M. Tudose, Deuterium content variation of human blood serum, Stud. Univ. Babes-Bolyai Phys. Spec. Issue (2001) 256–258.
[6] U. Zimermann, U. Cegla, Deuterium and oxygen-18 contents in the body fluids of man and their deviations due to change of location, Naturwissenschaften 60 (1973) 243–246.
[7] A. Yarat, Ö. Saçan, T. Tunalı Akbay, R. Yanardağ, Türkiye'de içme sularında döteryum konsantrasyonu (in Turkish) (Deuterium concentration in drinking water in Turkey), DSI Tek. Bülteni (DSI Tech. Bull.) 120 (2016) 35–40.
[8] D.S. Ávila, G. Somlyai, I. Somlyai, M.M. Aschner, Anti-aging effects of deuterium depletion on Mn-induced toxicity in a *C. elegans* model, Toxicol. Lett. 211 (3) (2012) 319–324, https://doi.org/10.1016/j.toxlet.2012.04.014.
[9] L.G. Boros, D.P. D'Agostino, H.E. Katz, J.P. Roth, E.J. Meuillet, G. Somlyai, Submolecular regulation of cell transformation by deuterium depleting water exchange reactions in the tricarboxylic acid substrate cycle, Med. Hypotheses 87 (2016) 69–74, https://doi.org/10.1016/j.mehy.2015.11.016.
[10] S.S. Dzhimak, A.A. Basov, M.G. Baryshev, Content of deuterium in biological fluids and organs: influence of deuterium depleted water on D/H gradient and the process of adaptation, Dokl. Biochem. Biophys. 465 (1) (2015) 370–373, https://doi.org/10.1134/S1607672915060071.
[11] Z. Gyöngyi, F. Budán, I. Szabó, I. Ember, I. Kiss, K. Krempels, G.M. Somlyai, Deuterium depleted water effects on survival of lung cancer patients and expression of Kras, Bcl2, and Myc genes in mouse lung, Nutr. Cancer 65 (2) (2013) 240–246, https://doi.org/10.1080/01635581.2013.756533.
[12] B.Z. Kovács, I. Somlyai, A. Papp, G. Somlyai, Deuterium-depleted water delayed hormone therapy of prostate cancer, J. Clin. Rev. Case Rep. 6 (10) (2021) 747–749.
[13] A.B. Lisicin, M.G. Baryshev, A.A. Basov, E.V. Barisheva, I.M. Bikov, A.S. Didikin, E. E. Tekutskaya, A.A. Timakov, L.V. Fedulova, I.M. Chernuha, S.S. Dzhimak, Influence of deuterium depleted water on the organism of laboratory animals in various functional conditions of nonspecific protective system, Biophysics 59 (4) (2014) 757–765, https://doi.org/10.1134/S0006350914040186.
[14] A.L. Luo, Research progress of biological effects of deuterium-depleted water, J. Shanghai Jiaotong Univ. (Med. Sci.) 12 (2018) 467–471.

[15] C. Mladin, A. Ciobica, R. Lefter, A. Popescu, W. Bild, Deuterium depleted water has stimulating effects on long-term memory in rats, Neurosci. Lett. 583 (2014) 154–158, https://doi.org/10.1016/j.neulet.2014.09.037.

[16] S. Soleyman-Jahi, K. Zendehdel, K. Akbarzahed, M. Haddadi, S. Amanpour, S. Muhammadnejad, *In vitro* assessment of antineoplastic effects of deuterium depleted water, Asian Pac. J. Cancer Prev. 15 (5) (2014) 2179–2183, https://doi.org/10.7314/APJCP.2014.15.5.2179.

[17] T. Strekalova, M. Evans, A. Chernopiatko, Y. Couch, J. Costa-Nunes, R. Cespuglio, L. Chesson, J. Vignisse, H.W. Steinbusch, D.C. Antony, I. Pomytkin, K.P. Lesch, Deuterium content of water increases depression susceptibility: the potential role of a serotonin-related mechanism, Behav. Brain Res. 277 (2015) 237–244, https://doi.org/10.1016/j.bbr.2014.07.039.

[18] S. Yılmaz, A. Tokpınar, E. Eroğlu, Ş. Ateş, R. Zahid, S. Avnioğlu, Ö. Bozkurt, M. Nisari, The effect of antioxidants in Ehrlich ascites cancer, Cell. Mol. Biol. 7 (2) (2021) 20–24, https://doi.org/10.14715/cmb/2021.67.2.4.

[19] R. Lawal, M. Ozaslan, Effect of medicinal plants on Ehrlich ascites carcinoma cells (A review), Zeugma Biol. Sci. 2 (2) (2021) 19–27.

[20] D.S. Sergeevich, B.A. Alexandrovich, E.A. Anatolyevna, F.L. Viacheslavovna, Influence of deuterium-depleted water on hepatorenal toxicity, Jundishapur J. Nat. Pharm. Prod. 13 (2) (2018), e69557, https://doi.org/10.5812/jjnpp.69557.

[21] F. Fatemi, A. Golbodagh, R. Hojihosseini, A. Dadkhah, K. Akbarzadeh, S. Dini, M.R. M. Malayeri, Anti-inflammatory effects of deuterium-depleted water plus *Rosa damascena* Mill. essential oil via cyclooxygenase-2 pathway in rats, Turk. J. Pharm. Sci. 17 (1) (2020) 99–107, https://doi.org/10.4274/tjps.galenos.2018.24381.

[22] A. Rasooli, F. Fatemi, R. Hajihosseini, A. Vaziri, K. Akbarzadeh, M.R.M. Malayeri, S. Dini, M. Foroutanrad, Synergistic effects of deuterium depleted water and *Mentha longifolia* L. essential oils on sepsis-induced liver injuries through regulation of cyclooxygenase-2, Pharm. Biol. 57 (1) (2019) 125–132, https://doi.org/10.1080/13880209.2018.1563622.

[23] E. Beutler. Glutathione in Red Cell Metabolism: A Manual of Biochemical Methods, second ed, Grune and Stratton, New York, 1975, pp. 112–114.

[24] A. Ledwozyw, J. Michalak, A. Stepien, A. Kadziolka, The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis, Clin. Chim. Acta 155 (3) (1986) 275–284, https://doi.org/10.1016/0009-8981(86)90247-0.

[25] L. Warren, The thiobarbituric acid assay of sialic acids, J. Biol. Chem. 234 (1959) 1971–1975, https://doi.org/10.1016/S0021-9258(18)69851-5.

[26] G.I. Ingram, M. Hills, Reference method for the one-stage prothrombin time test on human blood. International committee for standardization in hematology, Thromb. Haemost. 36 (1) (1976) 237–238, https://doi.org/10.1055/s-0038-1648029.

[27] H. Aebi, Catalase in vitro, Method. Enzymol. 105 (1984) 121–126, https://doi.org/10.1016/S0076-6879(84)05016-3.

[28] A.A. Mylroie, H. Collins, C. Umbles, J. Kyle, Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate, Toxicol. Appl. Pharmacol. 82 (3) (1986) 512–520, https://doi.org/10.1016/0041-008X(86)90286-3.

[29] D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70 (1) (1967) 158–169, https://doi.org/10.5555/uri:pii:0022214367900765.

[30] A. Wendel, Glutathione peroxidase, Method. Enzymol. 77 (1981) 325–333, https://doi.org/10.1016/S0076-6879(81)77046-0.

[31] E. Beutler, Red Cell Metabolism: A Manual of Biochemical Methods, vol. 12, Academic Press, London, 1971, pp. 68–70.

[32] W.H. Habig, W.B. Jakoby, Assays for differentiation of glutathione S-transferases, Method. Enzymol. 77 (1981) 398–405, https://doi.org/10.1016/s0076-6879(81)77053-8.

[33] G. Szasz, A kinetic photometric method for serum gamma-glutamyl transpeptidase, Clin. Chem. 15 (2) (1969) 124–136, https://doi.org/10.1093/clinchem/15.2.124.

[34] H. Wei, K. Frenkel, *In vivo* formation of oxidized DNA bases in tumor promoter-treated mouse skin, Cancer Res. 51 (16) (1991) 4443–4449.

[35] E.D. Corte, F. Stirpe, Regulation of xanthine oxidase in rat liver: modifications of the enzyme activity of rat liver supernatant on storage at 20 degrees, Biochem. J. 108 (2) (1968) 349–351, https://doi.org/10.1042/bj1080349.

[36] A.S. Ridderstap, S.L. Bonting, Na^{+}-K^{+}-activated ATPase and exocrine pancreatic secretion in vitro, Am. J. Physiol. 217 (6) (1969) 1721–1727, https://doi.org/10.1152/ajplegacy.1969.217.6.1721.

[37] C.E. Furlong, R.J. Richter, S.J. Seidel, Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon, Am. J. Hum. Genet. 43 (3) (1988) 230–238.

[38] R.L. Levine, D. Garland, C.N. Oliver, A. Amici, I. Climent, A.G. Lenz, B.B. Ahn, S. Shaltiel, E.R. Stadtman, Determination of carbonyl content in oxidatively modified proteins, Method. Enzymol. 186 (1990) 464–478, https://doi.org/10.1016/0076-6879(90)86141-H.

[39] O.C.O. Barretto, E. Beutler, The sorbitol-oxidizing enzyme of red blood cells, J. Lab. Clin. Med. 85 (4) (1975) 645–649, https://doi.org/10.5555/uri:pii:0022214375904977.

[40] O.H. Lowry, N.J. Rosebrough, A.R. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275, https://doi.org/10.1016/S0021-9258(19)52451-6.

[41] B. Alotaibi, E. Tousson, T.A. El-Masry, N. Altwaijry, A. Saleh, Ehrlich ascites carcinoma as model for studying the cardiac protective effects of curcumin nanoparticles against cardiac damage in female mice, Environ. Toxicol. 36 (1) (2021) 105–113, https://doi.org/10.1002/tox.23016.

[42] M. Ozaslan, I.D. Karagoz, I.H. Kilic, M.E. Guldur, Ehrlich ascites carcinoma, Afr. J. Biotechnol. 10 (13) (2011) 2375–2378.

[43] V.R. Bovilla, P.G. Anantharaju, S. Dornadula, P.M. Veeresh, M.G. Kuruburu, V. G. Bettada, K.M. Ramkumar, S.L. Madhunapantula, Caffeic acid and protocatechuic acid modulate Nrf2 and inhibit Ehrlich ascites carcinomas in mice, Asian Pac. J. Trop. Biomed. 11 (6) (2021) 244–256, https://doi.org/10.4103/2221-1691.314045.

[44] S.S. Agrawal, S. Saraswati, R. Mathur, M. Pandey, Antitumor properties of Boswellic acid against Ehrlich ascites cells bearing mouse, Food Chem. Toxicol. 49 (9) (2011) 1924–1934, https://doi.org/10.1016/j.fct.2011.04.007.

[45] A.L.D. Melo, M.C. Marcucci, C.D. Carvalho, Immunomodulatory activity in tumor-bearing mice treated with *Withania somnifera* extract, J. Anal. Pharm. Res. 10 (2) (2021) 82–91.

[46] A.V. Naik, S.N. Dessai, K. Sellappan, Antitumour activity of Annona muricata L. leaf methanol extracts against Ehrlich Ascites Carcinoma and Dalton's Lymphoma Ascites mediated tumours in Swiss albino mice, Libyan J. Med. 16 (1) (2021), 1846862, https://doi.org/10.1080/19932820.2020.1846862.

[47] A. Bansal, M.C. Simon, Glutathione metabolism in cancer progression and treatment resistance, J. Cell. Biol. 217 (7) (2018) 2291–2298, https://doi.org/10.1083/jcb.201804161.

[48] S. Spampinato, G. Sferrazzo, V. Pittalà, M. Di Rosa, L. Vanella, L. Salerno, V. Sorrenti, G. Carota, N. Parrinello, M. Raffaele, D. Tibullo, G. Li Volti, I. Barbagallo, Non-competitive heme oxygenase-1 activity inhibitor reduces non-small cell lung cancer glutathione content and regulates cell proliferation, Mol. Biol. Rep. 47 (2020) 1949–1964, https://doi.org/10.1007/s11033-020-05292-y.

[49] H.J. Forman, H. Zhang, A. Rinna, Glutathione: overview of its protective roles, measurement, and biosynthesis, Mol. Asp. Med. 30 (1–2) (2009) 1–12, https://doi.org/10.1016/j.mam.2008.08.006.

[50] E. Fadillioğlu, H. Erdoğan, S. Söğüt, I. Kuku, Protective effects of erdosteine against doxorubicin-induced cardiomyopathy in rats, J. Appl. Toxicol. 23 (1) (2003) 71–74, https://doi.org/10.1002/jat.889.

[51] H.R. Yilmaz, E. Uz, N. Yucel, I. Altuntas, N. Ozcelik, Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver, J. Biochem. Mol. Toxicol. 18 (4) (2004) 234–238, https://doi.org/10.1002/jbt.20028.

[52] S.V. Mantha, M. Prasad, J. Kalra, K. Prasad, Antioxidant enzymes in hypercholesterolemia and effects of vitamin E in rabbits, Atherosclerosis 101 (2) (1993) 135–144, https://doi.org/10.1016/0021-9150(93)90110-G.

[53] B.B. Bayrak, P. Koroglu, O. Karabulut Bulan, R. Yanardag, Metformin protects against diabetes-induced heart injury and dunning prostate cancer model, Hum. Exp. Toxicol. 40 (2) (2021) 297–309, https://doi.org/10.1177/0960327120947452.

[54] P. Koroglu-Aydın, B.B. Bayrak, I. Bugan, O. Karabulut-Bulan, R. Yanardag, Histological and biochemical investigation of the renoprotective effects of metformin in diabetic and prostate cancer model, Toxicol. Mech. Method. 31 (7) (2021) 489–500, https://doi.org/10.1080/15376516.2021.1919810.

[55] I.B. Turkyilmaz, B. Bilgin Sokmen, R. Yanardag, Alpha-lipoic acid prevents brain injury in rats administered with valproic acid, J. Biochem. Mol. Toxicol. 34 (11) (2020), e22580, https://doi.org/10.1002/jbt.22580.

[56] H. Kappus, Lipid peroxidation: mechanism, analysis, enzymology and biologic relevance, in: S. Helmut (Ed.), Oxidative Stress, Academic Press, London, 1985, pp. 273–310.

[57] N. Mackman, Tissue-specific hemostasis in mice, Arterioscler. Thromb. Vasc. Biol. 25 (11) (2005) 2273–2281, https://doi.org/10.1161/01.ATV.0000183884.06371.52.

[58] H.E. Wagner, P. Thomas, B.C. Wolf, A. Rapoza, G. Steele Jr, Inhibition of sialic acid incorporation prevents hepatic metastases, Arch. Surg. 125 (3) (1990) 351–354, https://doi.org/10.1001/archsurg.1990.01410150073013.

[59] J.E. Mrochek, S.R. Dinsmore, D.C. Tormey, T.P. Waalkes, Protein-bound carbohydrates in breast cancer. liquid-chromatographic analysis for mannose, galactose, fructose, and sialic acid in serum, Clin. Chem. 22 (9) (1976) 1516–1521, https://doi.org/10.1093/clinchem/22.9.1516.

[60] M.J. Vilarem, J. Jouanneau, R. Bourrillon, Differences in sialic acid contents of low cancer cells, high cancer cells and normal mouse lung counterparts, Biochem. Biophys. Res. Commun. 98 (1) (1981) 7–14, https://doi.org/10.1016/0006-291X(81)91862-3.

[61] G. Pizzino, N. Irrera, M. Cucinotta, G. Pallio, F. Mannino, V. Arcoraci, F. Squadrito, D. Altavilla, A. Bitto, Oxidative stress: harms and benefits for human health, 13 pages, Oxid. Med. Cell. Longev. 2017 (2017), 8416763, https://doi.org/10.1155/2017/8416763.

[62] I. Marrocco, F. Altieri, I. Peluso, Measurement and clinical significance of biomarkers of oxidative stress in humans, 32 pages, Oxid. Med. Cell. Longev. 2017 (2017), 6501046, https://doi.org/10.1155/2017/6501046.

[63] A. Rasooli, F. Fatemi, K. Akbarzadeh, S. Dini, S.H. Bahremand, Synergistic protective activity of deuterium depleted water (DDW) and *Satureja rechingeri* essential oil on hepatic oxidative injuries induced by acetaminophen in rats, J. Essent. Oil-Bear. Plants 19 (5) (2016) 1086–1101, https://doi.org/10.1080/0972060X.2015.1111776.

[64] L. Olariu, M. Petcu, C. Tulcan, I. Chis-Buiga, M. Pup, M. Florin, I. Brudiu, Deuterium depleted water – antioxidant or prooxidant? Lucr. Stiinlifice Med. Vet. Timis. 11 (2004) 265–269.

[65] S. Yardım Akaydın, E. Miser Salihoğlu, D. Gelen Güngör, H. Karanlık, S. Demokan, Correlation between gamma-glutamyl transferase activity and glutathione levels in molecular subgroups of breast cancer, Eur. J. Breast Health 16 (1) (2020) 72–76, https://doi.org/10.5152/ejbh.2019.5147.

[66] S. Hendrich, H.V. Pitot, Enzymes of glutathione metabolism as biochemical markers during hepatocarcinogenesis, Cancer Metastas. Rev. 6 (2) (1987) 155–178, https://doi.org/10.1007/BF00052847.

[67] H. Qian, X. Chao, J. Williams, S. Fulte, T. Li, L. Yang, W.X. Ding, Autophagy in liver diseases: a review, Mol. Asp. Med. 82 (2021), 100973, https://doi.org/10.1016/j.mam.2021.100973.
[68] E. Gilbert-Barness, L.A. Barness, P.M. Farrell, Liver disease. Clinical Use of Pediatric Diagnostic Tests, sixth ed, Lippincott, Williams and Wilkins, Philadelphia, 2003, pp. 303–326.
[69] G. Ndrepepa, Aspartate aminotransferase and cardiovascular disease-a narrative review, J. Lab. Precis. Med. 6 (2021) 8725, https://doi.org/10.21037/jlpm-20-93.
[70] X. Zhang, M. Gaetani, A. Chernobrovkin, R.A. Zubarev, Anticancer effect of deuterium depleted water - redox disbalance leads to oxidative stress, Mol. Cell. Proteom. 18 (12) (2019) 2373–2387, https://doi.org/10.1074/mcp.RA119.001455.
[71] D.M. Parkin, P. Pisani, N. Muñoz, J. Ferlay, Infections and Human Cancer, Cold Spring Harbor Laboratory Press, New York, 1999.
[72] D. Lau, H. Mollnau, J.P. Eiserich, B.A. Freeman, A. Daiber, U.M. Gehling, J. Brümmer, V. Rudolph, T. Münzel, T. Heitzer, T. Meinertz, S. Baldus, Myeloperoxidase mediates neutrophil activation by association with CD11b/CD18 integrins, Proc. Natl. Acad. Sci. USA 102 (2) (2005) 431–436, https://doi.org/10.1073/pnas.0405193102.
[73] J. Zeindler, F. Angehrn, R. Droeser, S. Däster, S. Piscuoglio, C.K.Y. Ng, E. Kilic, R. Mechara, S. Meili, A. Isaak, W.P. Weber, S. Muenst, D.S. Soysal, Infiltration by myeloperoxidase-positive neutrophils is an independent prognostic factor in breast cancer, Breast Cancer Res. Treat. 177 (2019) 581–589, https://doi.org/10.1007/s10549-019-05336-3.
[74] M.G. Battelli, L. Polito, M. Bortolotti, A. Bolognesi, Xanthine oxidoreductase in cancer: more than a differentiation marker, Cancer Med. 5 (3) (2016) 546–557, https://doi.org/10.1002/cam4.601.
[75] A. Veljković, J. Hadži-Dokić, D. Sokolović, D. Bašić, L. Velićković-Janković, J. W. Shay, A. Tiwari, F. Buffoli, E. Laczko, G. Marra, Xanthine oxidase/dehydrogenase activity as a source of oxidative stress in prostate cancer tissue, Diagnostics 10 (9) (2020) 668, https://doi.org/10.3390/diagnostics10090668.
[76] J. Tian, T. Cai, Z. Yuan, H. Wang, L. Liu, M. Haas, E. Maksimova, X.Y. Huang, Z. J. Xie, Binding of Src to Na^{+}/K^{+}-ATPase forms a functional signaling complex, Mol. Biol. Cell. 17 (2006) 317–326, https://doi.org/10.1091/mbc.e05-08-0735.
[77] A. Aydemir-Koksoy, J. Abramowitz, J.C. Allen, Ouabain-induced signaling and vascular smooth muscle cell proliferation, J. Biol. Chem. 276 (2021) 46605–46611, https://doi.org/10.1074/jbc.M106178200.
[78] L. Chen, P. Jiang, J. Li, Z. Xie, Y. Xu, W. Qu, F. Feng, W. Liu, Periplocin promotes wound healing through the activation of Src/ERK and PI3K/Akt pathways mediated by Na/K-ATPase, Phytomedicine 57 (2019) 72–83, https://doi.org/10.1016/j.phymed.2018.12.015.
[79] M.A. Khajah, P.M. Mathew, Y.A. Luqmani, Na^{+}/K^{+} ATPase activity promotes invasion of endocrine resistant breast cancer cells, PLoS One 13 (2018), e0193779, https://doi.org/10.1371/journal.pone.0193779.
[80] J. Bejček, V. Spiwok, E. Kmoníčková, S. Rimpelová, Na^{+}/K^{+}-ATPase revisited: on its mechanism of action, role in cancer, and activity modulation, Molecules 26 (7) (2021) 1905, https://doi.org/10.3390/molecules26071905.
[81] P. Babula, M. Masarik, V. Adam, I. Provaznik, R. Kizek, From Na^{+}/K^{+}-ATPase and cardiac glycosides to cytotoxicity and cancer treatment, Anticancer Agents Med. Chem. 13 (7) (2013) 1069–1087, https://doi.org/10.2174/18715206113139990304.
[82] G.A. Figtree, C.C. Liu, S. Bibert, E.J. Hamilton, A. Garcia, C.N. White, K.K. Chia, F. Cornelius, K. Geering, H.H. Rasmussen, Reversible oxidative modification: a key mechanism of Na^{+}-K^{+} pump regulation, Circ. Res. 105 (2) (2009) 185–193, https://doi.org/10.1161/CIRCRESAHA.109.199547.
[83] S.T. Reddy, A. Devarajan, N. Bourquard, D. Shih, A.M. Fogelman, Is it just paraoxonase 1 or are other members of the paraoxonase gene family implicated in atherosclerosis? Curr. Opin. Lipidol. 19 (2008) 405–408, https://doi.org/10.1097/MOL.0b013e328304b64e.
[84] Y. Jin, Y. Yang, Y. Su, X. Ye, W. Liu, Q. Yang, J. Wang, X. Fu, Y. Gong, H. Su, Identification a novel clinical biomarker in early diagnosis of human non-small cell lung cancer, Glycoconj. J. 36 (2019) 57–68, https://doi.org/10.1007/s10719-018-09853-z.
[85] P. Wigner, B. Szymańska, M. Bijak, E. Sawicka, P. Kowal, Z. Marchewka, J. Saluk-Bijak, Oxidative stress parameters as biomarkers of bladder cancer development and progression, Sci. Rep. 11 (1) (2021) 15134, https://doi.org/10.1038/s41598-021-94729-w.
[86] A.M. Pisoschi, A. Pop, F. Iordache, L. Stanca, G. Predoi, A.I. Serban, Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status, Eur. J. Med. Chem. 209 (2021), 112891, https://doi.org/10.1016/j.ejmech.2020.112891.
[87] R. Ahmad, A.K. Tripathi, P. Tripathi, S. Singh, R. Singh, R.K. Singh, Malondialdehyde and protein carbonyl as biomarkers for oxidative stress and disease progression in patients with chronic myeloid leukemia, In Vivo 22 (2008) 525–528.
[88] A.A. Basov, A.A. Elkina, A.A. Samkov, N.N. Volchenko, A.V. Moiseev, L. V. Fedulova, M.G. Barysev, S.S. Dzhimak, Influence of deuterium-depleted water on the isotope D/H composition of liver tissue and morphological development of rats at different periods of ontogenesis, Iran. Biomed. J. 23 (2) (2019) 129–141, https://doi.org/10.29252/.23.2.129.
[89] A. Uzozie, P. Nanni, T. Staiano, J. Grossmann, S. Barkow-Oesterreicher, J.W. Shay, A. Tiwari, F. Buffoli, E. Laczko, G. Marra, Sorbitol dehydrogenase overexpression and other aspects of dysregulated protein expression in human precancerous colorectal neoplasms: a quantitative proteomics study, Mol. Cell. Proteom. 13 (5) (2014) 1198–1218, https://doi.org/10.1074/mcp.M113.035105.