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Original Article

Ameliorative potential role of *Rosmarinus officinalis* extract on toxicity induced by etoposide in male albino rats

Papel potencial de melhora do extrato de *Rosmarinus officinalis* na toxicidade induzida por etoposídeo em ratos albinos machos

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Abstract

The present work was showed to assess the effect of administration of rosemary extract on etoposide-induced toxicity, injury and proliferation in male rats were investigated. Forty male albino rats were arranged into four equal groups. 1st group, control; 2nd group, etoposide; 3rd group, co-treated rosemary & etoposide; 4th group, rosemary alone. In comparison to the control group, etoposide administration resulted in a significant increase in serum ALT, AST, ALP, total bilirubin, total protein, and gamma GT. In contrast; a significant decrease in albumin level in etoposide group as compared to G1. G3 revealed a significant decrease in AST, ALT, ALP, total protein and total bilirubin levels and a significant rise in albumin level when compared with G2. Serum levels of urea, creatinine, potassium ions, and chloride ions significantly increased; while sodium ions were significantly decreased in G2 when compared with G1. Also, there was an increase of MDA level for etoposide treated group with corresponding control rats. However, there was a remarkable significant decrease in SOD, GPX and CAT levels in G2 as compared to G1. There was a significant increase in serum hydrogen peroxide (H_2O_2) and Nitric oxide (NO) levels in group treated with etoposide when compared to control group. It was noticeable that administrated by rosemary alone either with etoposide had not any effect on the levels of H_2O_2 and Nitric oxide. Serum level of T3 and T4 was significantly increased in etoposide-administered rats in comparison with G1. The administration of rosemary, either alone or with etoposide, increased the serum levels of T3 and T4 significantly when compared to control rats. The gene expression analysis showed significant downregulation of hepatic SOD and GPx in (G2) when compared with (G1). The treatment with rosemary extract produced significant upregulation of the antioxidant enzymes mRNA SOD and GPx. MDA gene was increased in (G2) when contrasted with (G1). Treatment of the etoposide- induced rats with rosemary extract delivered significant decrease in MDA gene expression when compared with etoposide group. Rats treated with etoposide showed significant decline in hepatic Nrf2 protein expression, when compared with G1. While, supplementation of Etoposide- administered rats with the rosemary produced a significant elevation in hepatic Nrf2 protein levels. Additionally, the liver histological structure displayed noticeable degeneration and cellular infiltration in liver cells. It is possible to infer that rosemary has a potential role and that it should be researched as a natural component for etoposide-induced toxicity protection.

Keywords: rosemary, etoposide, chemotherapy, liver, rat.

Resumo

O presente trabalho foi apresentado para avaliar o efeito da administração de extrato de alecrim na toxicidade, lesão e proliferação induzidas por etoposídeos em ratos machos. Quarenta ratos albinos machos foram organizados em quatro grupos iguais: 1º grupo, controle; 2º grupo, etoposídeo; 3º grupo, alecrim e etoposídeo cotratados; 4º grupo, alecrim sozinho. Em comparação com o grupo controle, a administração de etoposídeo resultou em aumento significativo da ALT, AST, ALP, bilirrubina total, proteína total e gama GT séricas. Em contraste, houve diminuição significativa do nível de albumina no grupo etoposídeo em relação ao G1. O G3 revelou diminuição significativa dos níveis de AST, ALT, ALP, proteína total e bilirrubina total e aumento significativo do nível de albumina quando comparado ao G2. Os níveis séricos de ureia, creatinina, íons potássio e íons cloreto aumentaram significativamente, enquanto os íons sódio diminuíram significativamente no G2 quando comparado ao G1. Além disso, houve um aumento do nível de MDA para o grupo tratado com etoposídeo com os ratos controle correspondentes. No entanto, houve uma notável diminuição nos níveis de SOD, GPX e CAT no G2 em relação ao G1. Houve aumento significativo dos níveis séricos de peróxido de hidrogênio (H_2O_2) e óxido nítrico (NO) no grupo tratado com etoposídeo quando comparado ao grupo controle. Foi perceptível que a administração de alecrim isoladamente ou com etoposídeo não teve efeito sobre os níveis de H_2O_2 e NO. O nível sérico de T3 e T4 foi significativamente aumentado em ratos administrados com etoposídeo em comparação com o G1. A administração de alecrim, isoladamente ou com etoposídeo, aumentou significativamente os níveis séricos de T3 e T4 quando comparada aos ratos controle. A

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análise da expressão gênica mostrou desregulação significante da SOD e GPx hepática em G2 quando comparado com o G1. O tratamento com extrato de alecrim produziu aumento significativo das enzimas antioxidantes mRNA SOD e GPx. O gene MDA estava aumentado em G2 quando contrastado com o G1. O tratamento dos ratos induzidos por etoposídeo com extrato de alecrim proporcionou diminuição significativa na expressão do gene MDA quando comparado ao grupo etoposídeo. Ratos tratados com etoposídeo apresentaram declínio significativo na expressão da proteína Nrf2 hepática quando comparados ao G1. Enquanto isso, a suplementação de ratos administrados com etoposídeo e alecrim produziu uma elevação significativa nos níveis de proteína hepática Nrf2. Além disso, a estrutura histológica do fígado apresentou degeneração perceptível e infiltração celular nas células hepáticas. É possível inferir que o alecrim tem um papel potencial e que deve ser pesquisado como um componente natural para proteção da toxicidade induzida por etoposídeos.

Palavras-chave: alecrim, etoposídeo, quimioterapia, fígado, rato.

Introduction

Chemotherapy is a term used to describe a group of medications used to treat cancer. As a result, finding medicines that can reduce anticancer medication side effects without reducing their efficacy or increasing toxicity or harm to target organs is crucial. Etoposide is a chemotherapeutic medication generated from Podophyllum peltatum, a plant (Al-Rasheed et al., 2017). Etoposide is a drug that is used to treat a variety of malignancies by inhibiting topoisomerase II activity, which causes normal growing cells to die. It also functions as a semi-synthetic molecule (Shin et al., 2016; Tousson et al., 2018).

Etoposide treatment cause severe hepato, nephron and testicular toxicity (Yuan et al., 2008). The alteration in liver, kidney and testis induced by anticancer drugs and closely associated with formation of reactive oxygen species in tissues. When organism cells are exposed to several enodogenous and oxogenous agents, reactive oxgen species are produced which in turn causes damage to nucleic acid, protein and lipid etc. To prevent damage from reactive oxygen species, cells possess different antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione s transferase (Kanchana et al., 2013).

Many plant extracts and products have been shown demonstrated to have high antioxidant activity, which could be a major property of medicinal plants used to treat a wide range of disorders, including liver damage (Saggu et al., 2014). Exogenous antioxidants found in medicinal plants may be a new alternative way to alleviate pathogenic changes in oxidative stress-related pathology (Oyouni et al., 2018).

Rosemary (Rosmarinus officinalis) is a spice that contains rosmarinic acid, caffeic acid, camphor, ursolic acid, betulinic acid, and carnosic acid, among other phytochemicals. Antitumor, antioxidant, antibacterial, antinociceptive, antidiabetic, antithrombotic, antiulcerogenic, antidiuretic, and anti-inflammatory agents are among the bioactivities it possesses in vitro (Mena et al., 2016; Shin et al., 2016). Because rosemary leaf extract contains flavonoids and phenols, it has potent antioxidant properties (Montecucco et al., 2015; Shin et al., 2016).

As a result, the aim of this investigation was to see if rosemary aqueous extract may help prevent etoposide-induced toxicity in male rats.

2. Materials and Methods

2.1. Animals

The experiments were carried out on a total of 20 male albino rats weighing 130–150 g and aged 9–10 weeks. The rats were kept in in plastic cages in the animals ' house of Nuclear Research Centre, Atomic Energy Authority and maintained on a standard rodent diet and water available ad libitum. Rats were separated into four groups after one week of acclimatisation.

2.2. Chemical

2.2.1. Rosemary extract

The rosemary extracts was prepared according to method described by Dorman et al. (2003).

2.2.2. Etoposide

Etoposide procured from Hikma specialized pharmaceuticals, Giza- Egypt.

2.3. Experimental groups

The male rats were divided into four groups, each with an equal number of rats. The first group, serves as a control group (G1), consisted of rats that were not given any therapy. Etoposide group (G2): For four weeks, rats were intraperitoneally injected with etoposide (1 mg/kg B.W/day). The third group (G3) consisted of rats that were given aqueous rosemary extract (220 mg/kg b.w./three times weekly) orally for four weeks and then injected with etoposide (1 mg/kg B.W./day) at the same time. Rosemary group (G4) rats were administered rosemary extract by oral gavage at a dosage of (220 mg/kg b.w./three times weekly) for four weeks.

Rats were lightly anesthetized at the end of the experiment, and fasting blood samples were taken by heart puncture after scarification. Blood was drawn into clean, dry test tubes and allowed to coagulate in a water bath at 37°C. Centrifugation at 3000 rpm for 10 minutes separated the serum. The supernatant serum was pipetted off with tipped automated pipettes and kept at -20°C until analysis (Wolfe, 2012).

2.4. Liver function biomarker

Liver enzymes alanine aminotransferase (ALT, Abcam ab105134), aspartate aminotransferase (AST, Abcam ab105135) and alkaline phosphatase (ALP, Abcam

ab83369) were evaluated using previous kits according to the manufacturer's protocols. Then, incubated for 5 min. finally, absorbance at 570 nm (ALT, AST) and 405 nm were determined according to the method of Schumann and Klauke (2003). (ALP) were determined according to Belfield and Goldberg (1971). Serum albumin was estimated according to Bradford (1976). While serum total proteins level was estimated according to Rolinski et al. (2001). Bilirubin Assay kit (abcam, ab235627) was estimated according to Whitfield (2001). Gamma glutmyl transferase was estimated according to Henery et al. (1974).

2.5. Electrolytes and kidney functions biomarker

Urea assay kit (abcam, ab83362) was measured according to the method described by Salama et al. (2013), creatinine Assay kit (abcam, ab204537) measured according to the method of Granger et al. (1996) and electrolytes according to Jain et al. (2009).

2.6. Assay of oxidative stress and antioxidant defense system

The content of lipid peroxidation in liver homogenates was determined by measurement of malondialdehyde (MDA) formation according to the method of Preuss et al. (1998). activities of the antioxidant enzymes, Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activity were determined using the techniques of Marklund and Marklund (1974), Matkovics (1988), and Cohen et al. (1970), respectively. Hydrogen peroxide assay kit (ab10250) measured according to the method of Fearon (1939) and nitric oxide Assay kit (abcam, ab65326) measured according to the method of Bartosz (2006).

2.7. Gene expression study

RNA was isolated via TRIzols Reagent (Thermo Fisher, USA), according to the user's protocol. Reverse transcription was constructed with QuantiTects Reverse Transcription Kit (Qiagen, USA), according to the user's manual. Then, sample purity was detected via Qubit 2.0 fluorometer. After *adding specific primers* in the qPCR reaction shown in Table 1. Conditions used for the reaction were previously described by Zhang et al. (2015) and Jardim-Goncalves et al. (2013). QPCR were performed on the Lightcycler 96 (Roche, Basel, Switzerland) thermocycler.

2.8. Western blot analysis

Purified sample proteins were loaded on a 10% SDS-PAGE (freshly prepared) was used to fragment purified protein samples by using omniPAGE Vertical Systems (Cleaver, UK) After protein separation, total proteins were transferred to a *Hybond*™ nylon membrane (GE Healthcare) via TE62 Standard Transfer Tank with Cooling Chamber (Hoefer Inc) and incubate for 1 hour at room temperature in Blocking Solution (5% dry milk). Anti-Nrf2 antibody (abcam, ab137550) was diluted as (1:1000) and Anti-beta Actin antibody [mAbcam 8226] was applied as housekeeping gene. Data visualized was performed via Gel documentation system (Geldoc-it, UVP, England) integrated with using Totallab analysis software, ww.totallab.com, (Ver.1.0.1)

2.9. Histopathological examinations

Following blood sample, rats were dissected, and the liver tissues were kept in 10% buffered formalin and fixed in paraffin immediately after being removed from the animals. After standard processing, each tissue was sliced into 4 µm thick paraffin slices and stained with hematoxylin and eosin. In furthermore, liver sections were stained with Masson's trichrome. All liver sections were examined and photographed using a light microscope.

2.10. Statistical analysis

Results are expressed as the mean ± standard deviation (SD). Statistical significant differences were evaluated using analysis of variance one way ANOVA using statistical package system software (SPSS software version 22) (Levesque, 2007).

3. Results

Figure 1 demonstrates that etoposide-treated rats had substantially higher serum ALT, AST, ALP, total protein, total bilirubin, and gamma GT levels than the control group($P<0.05$). Albumin levels in the etoposide-treated rats, on the other hand, were substantially lower ($P<0.05$) than in the control group. There was a significant ($P<0.05$) decrease in ALT, AST, ALP, total protein, and total bilirubin levels, as well as a significant($P<0.05$)rise in albumin levels, when comparing treated rats with etoposide and rosemary.

Table 1. Candidate genes.

Gene		Sequences	Reference
(SOD)	forward	5′-ACTGGTGGTCCATGAAAAAGC-3′	Zhang et al., 2015
	reverse	5′-AACGACTTCCAGCGTTTCCT-3′	
(GAPDH)	forward	5′-GAAGGTGAAGGTCGGAGTC-3′	
	reverse	5′-GAAGATGGTGATGGGATTTC-3′	
(GPX)	forward	5′-ATGGCGCAATTGTCCAAG-3′	Jardim-Goncalves et al., 2013
	reverse	5′-CTGGCC TCCCCTTACAGTG-3′	
(MDA)	forward	GGCTGCAGTAGGAAGTGGACAG	Carneiro et al., 2009
	reverse	GGTGAGGCCCAATGCACAA	

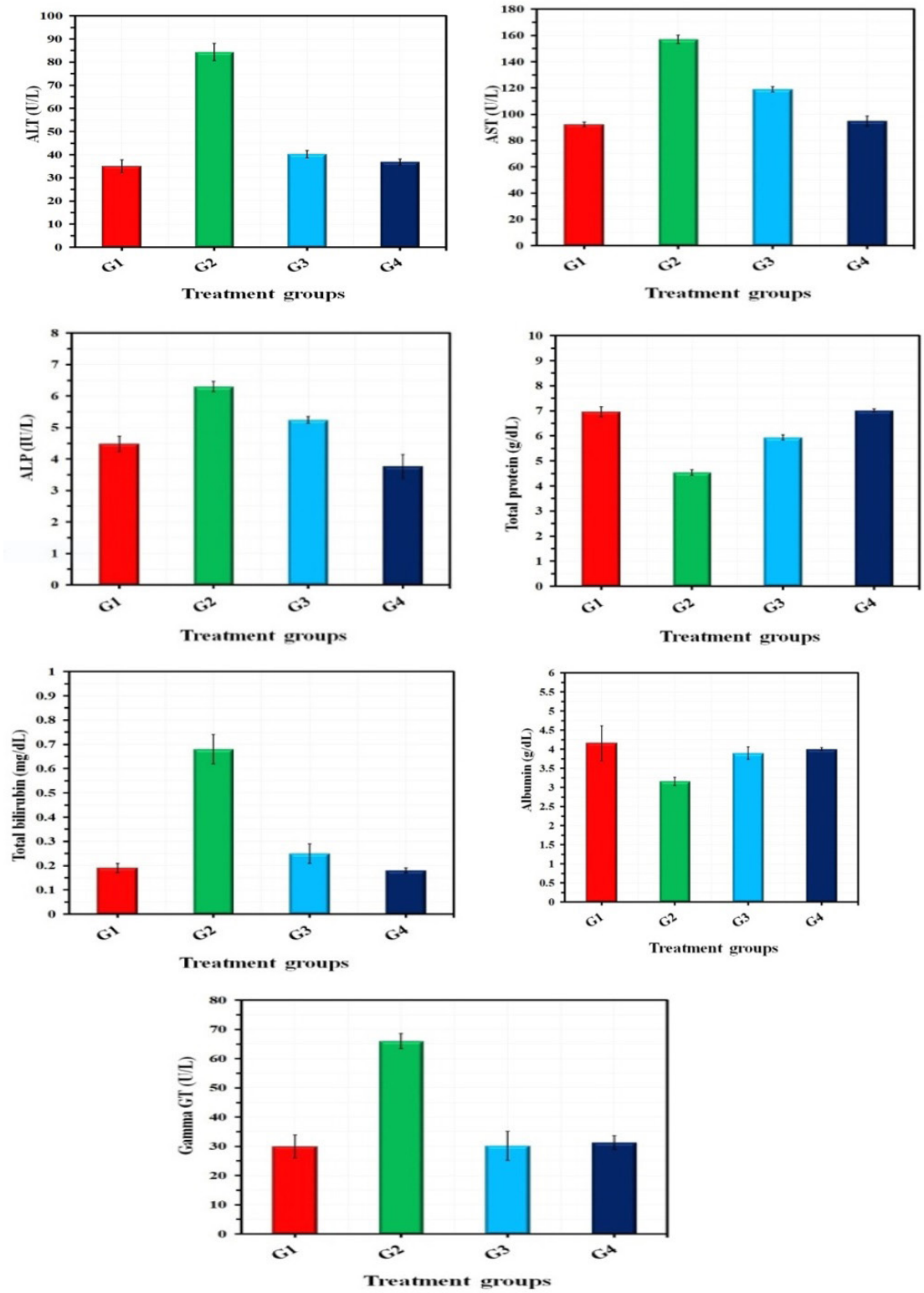

Figure 1. Effect of etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) administration on serum alanine amino transferase ALT (U/I), serum aspartate amino transferase AST (U/L), serum alkaline phosphatase ALP (IU/L), total protein(g/dl), total bilirubin, albumin and Gamma Glutamyl Transferase (GGT) on male albino rats. Data were expressed as means ±SE.

The serum urea, creatinine, potassium, and chloride ions levels in treated rats with etoposide were considerably ($P < 0.05$) higher than in the control group, according to data shown in Table 2. In contrast, etoposide-treated rats had a significant ($P < 0.05$) decrease in serum sodium level when compared to the control group. When rats were administered etoposide with rosemary (as in G4), urea, creatinine, potassium, and chloride ions decreased significantly ($P < 0.05$), while sodium levels increased significantly ($P < 0.05$).

Figure 2 depicts that there was an increase of MDA level for etoposide treated group with corresponding control rats. On the other hand, no significant change in MDA level was observed between (G1) and rosemary& etoposide treated groups (G3), rosematy treated group (G4). However, there was a remarkable significant decrease in SOD, GPX and CAT levels in G2 as compared to G1. Oral supplementation of rosemary increased the activity of SOD, GPx and CAT ($P<0.05$) as compared with the etoposide -administered group.

Data in Figure 3 postulated that there was a significant increase in serum hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels in etoposide group (G2) when compared to control group (G1). administration of rosemary to rats

Table 2. Effect of etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) administration on serum urea (mg/dl), serum creatinine (mg/dl) levels, potassium (K) (mmol/l), sodium (Na) (mmol/l) and chloride (Cl) (mmol/l) ions levels on male albino rats.

Parameters	G1	G2	G3	G4
Urea (mg/dl)	33.28±1.51^b	65.62±3.31^a	32.96±0.98^b	32.28±0.66^b
Creatinine (mg/dl)	0.65±0.06^b	2.83±0.28^a	0.77±0.04^b	0.63±0.02^b
K (mmol/l)	4.92±0.24^c	6.74±0.29^a	5.40±0.23^b	4.90±0.16^c
Na (mmol/l)	146.1±3.67^a	122.8±2.18^c	137.2±1.47^b	135.4±2.17^b
Chloride (mmol/l)	97.6±1.16^c	134±1.85^a	101±1.50^b	98.8±0.86^c

Data were expressed as means ± SD (standard deviation); (a,b,c…..etc): means bearing difference superscripts within the same row are significantly different at ($p<0.05$).

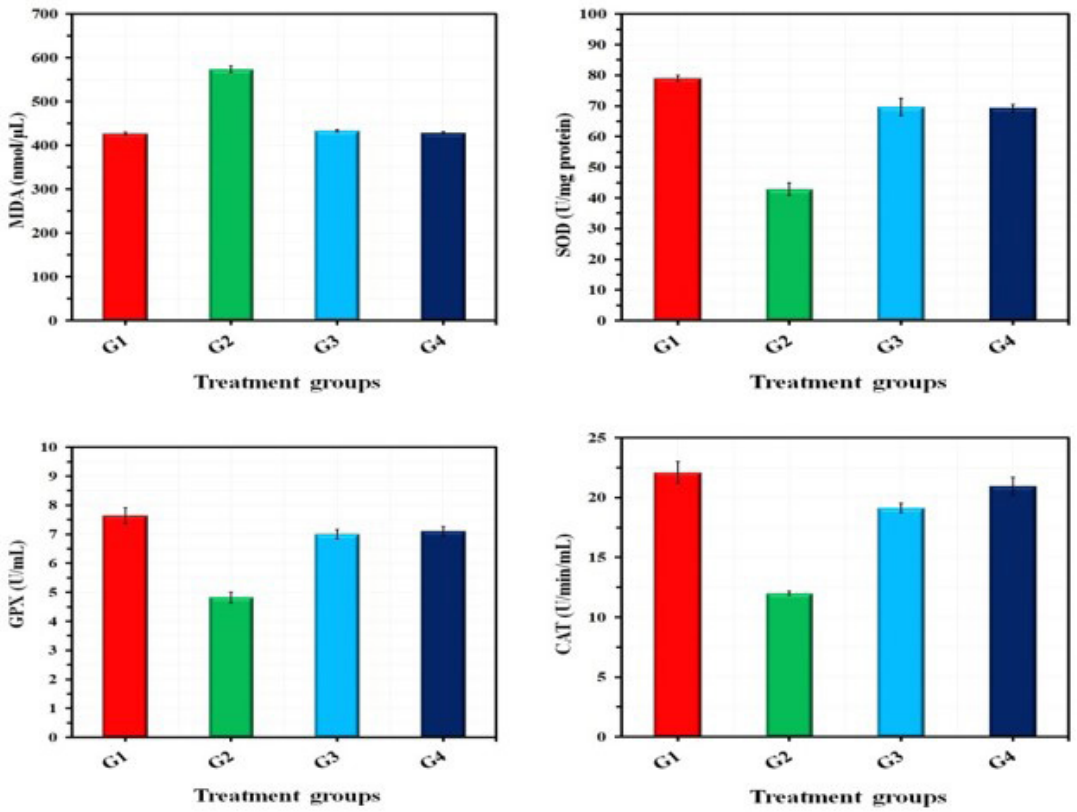

Figure 2. Effect of etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) administration on serum malondialdhyde (MDA) (nmol/µl), superoxide dismutase (SOD) (U/mg of protein), glutathione perioxidase (GPX) (U/ml), catalase (CAT) (U/min/ml) on male albino rats. Data were expressed as means ±SE.

(P<0.05) ameliorated the levels of (H_2O_2) and NO. It was noticeable that administrated by rosemary alone had not any effect on the levels of H_2O_2 and nitric oxide when compared with control group.

Serum level of triiodothyronine (T3) and thyroxine (T4) was significantly (P<0.01) increased in etoposide group in comparison with the control rats. The administration of rosemary, either alone or with etoposide, increased the serum levels of triiodothyronine (T3) and thyroxine (T4) significantly (P<0.01) when compared to control rats (Table 3).

3.1. Effect of rosemary extract on SOD, GPx, and MDA gene expression

The gene expression analysis showed significant downregulation of hepatic SOD (2^-ΔΔCt=0.310) and GPx (2^-ΔΔCt=0.664 in etoposide-intoxicated rats when compared with control group (G1). The treatment of the rosemary extract to rats (G3) produced significant (2^-ΔΔCt= 0.501 and 0.996) upregulation of the antioxidant enzymes mRNA SOD and GPx respectively (Figures 4-5).

Gene expression analysis showed significant elevation of hepatic MDA in etoposide treated rats when compared with the controls rats. there was a remarkable significant decrease MDA gene expression in etoposide and rosemary group (G3). As etoposide-induced rats were given rosemary extract, MDA gene expression was significantly reduced when compared to the etoposide-treated rats (Figure 6).

3.2. Effect of rosemary extract on Nrf2 protein expression

When compared to control rats, etoposide-administered rats had a significant decrease in hepatic Nrf2 protein expression (42.56 percent), as indicated by western blotting (Figure 7). The rosemary extract supplementation of etoposide-administered rats resulted in a significant increase (54.58 percent) in hepatic Nrf2 protein levels.

3.3. Liver histopathology

Histological examination of the liver sections obtained from control and rosemary groups revealed normal characteristic hepatic architecture where hepatic cords radiating from central vein with interfering blood sinusoid. The hepatocytes appeared as polygonal cell having rounded vesicular basophilic nuclei with prominent nucleoli and acidophilic cytoplasm. The portal area containing portal triads; branch of the portal vein, branch of the hepatic artery and in addition to a bile ductile (Figure 8A-B). Liver sections from Etoposide group revealed pronounced abnormalities in the form of vacuolar degeneration of some hepatocytes that appeared with dark stained nuclei with vacuolated cytoplasm and some hepatocytes were apoptotic with pyknotic nuclei that were deeply stained. Extensive lymphocytic cellular infiltration beside congestion and dilation of central, portal veins in addition to blood sinusoids were observed. Also proliferation of bile duct was detected (Figure 8C-F). Liver sections from rats treated with Etoposide with rosemary group revealed a marked improvement as most of hepatocytes t exhibited

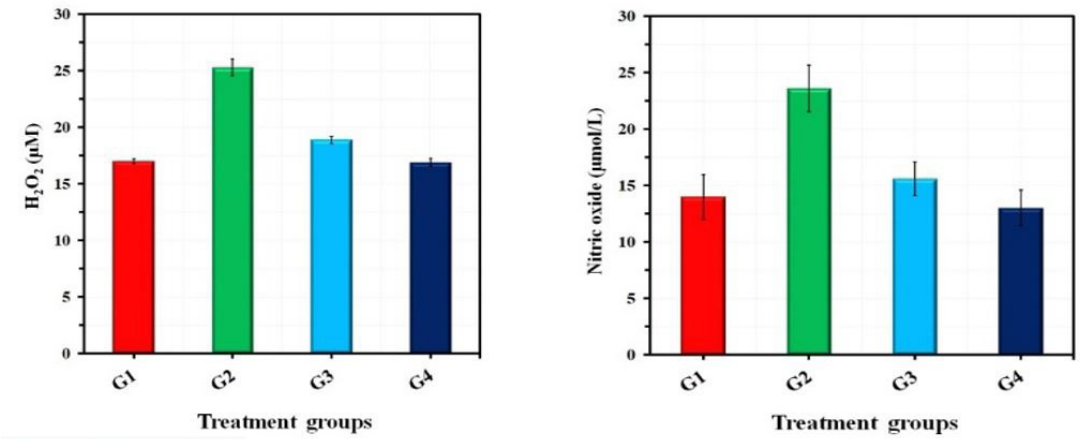

Figure 3. Effect of etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) administration on serum hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels of male albino rats. Data were expressed as means ±SE.

Table 3. Effect of etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) administration on serum triiodothyronine (T3) and thyroxine (T4) level of male albino rats.

Parameters	G1	G2	G3	G4
T3 level (pg/dl)	401.60±8.20^{d}	568.80±7.73^{b}	604.80±10.99^{a}	498.80±5.17^{c}
T4 level (ng/dl)	1.16±0.31^{c}	2.04±0.11^{b}	2.78±0.26^{a}	1.86±0.17^{b}

Data were expressed as means ± SD (standard deviation); (a,b,c…..etc): means bearing difference superscripts within the same row are significantly different at (p<0.05).

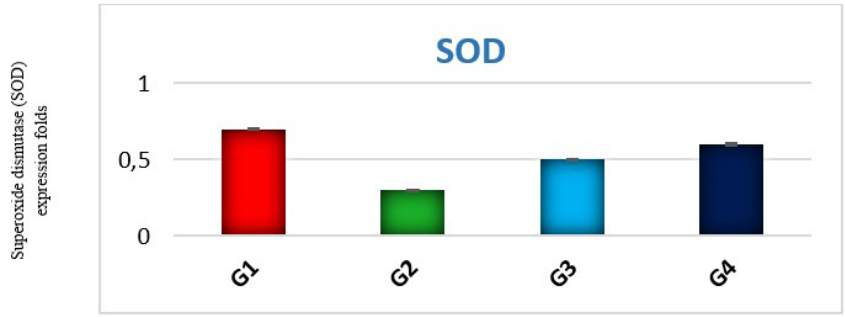

Figure 4. Superoxide dismutase (SOD) gene expression level for control (G1), etoposide (G2), etoposide & rosemary (G3) and rosemary alone (G4) groups. Data were expressed as means ±SE.

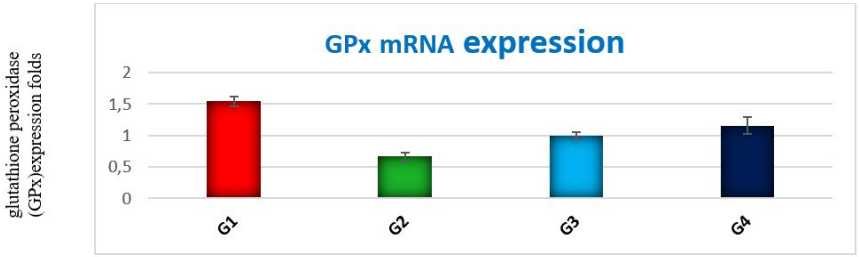

Figure 5. Glutathione peroxidase (GPx) gene expression level for control (G1), etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) groups. Data were expressed as means ±SE.

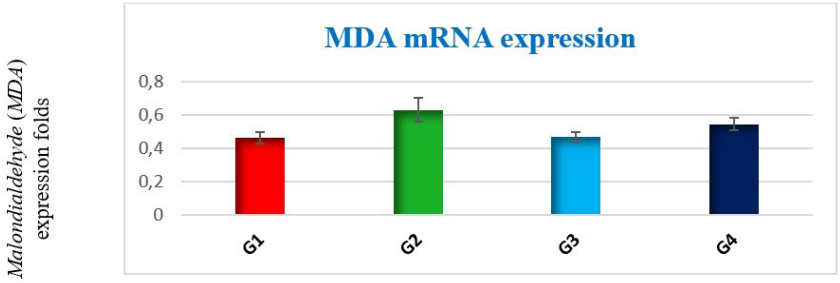

Figure 6. *Malondialdehyde* (*MDA*) gene expression level for control (G1), etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) groups. Data were expressed as means ±SE.

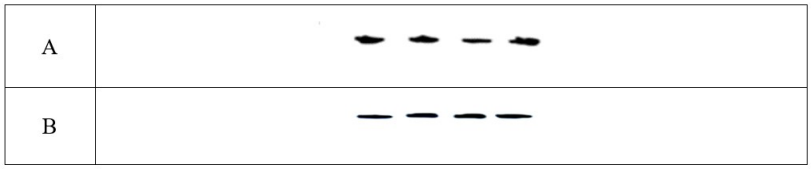

Figure 7. Nrf2 protein expression level (A) and β actin protein expression level (B) for control (G1), etoposide (G2), etoposide&rosemary (G3) and rosemary alone (G4) groups. Histopathological changes.

normal appearance however some hepatocytes still had pyknotic nuclei and scanty amount of hepatocytes were still observed vacuolated. Disappearance of sinusoid congestion was exhibited (Figure 8G-H).

4. Discussion

Tumors have spread these days and different types of chemotherapy are used to treat them. Chemotherapy uses chemical agents to eliminate cancer cells, whether

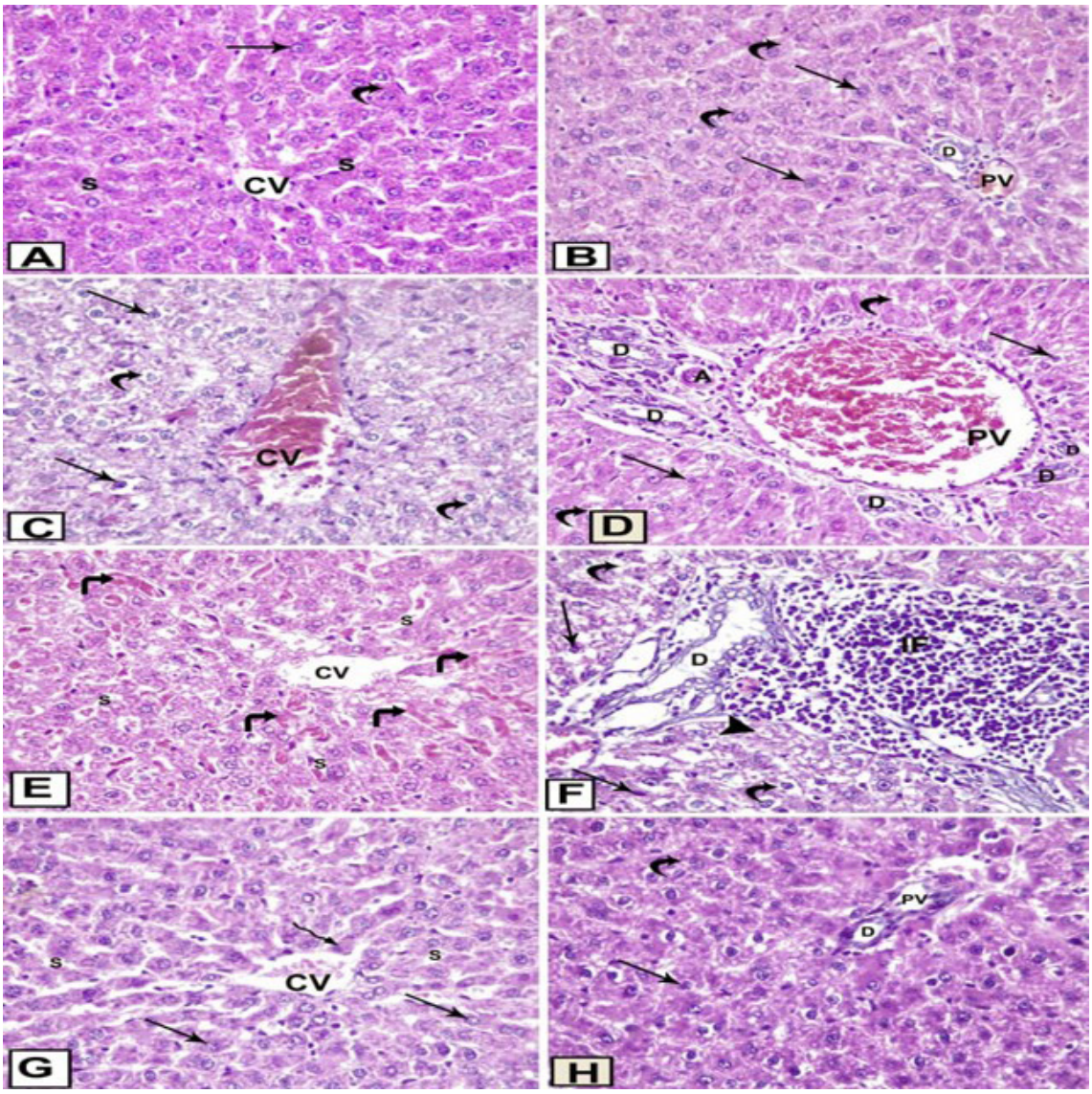

Figure 8. A-H: Photomicrographs of rat liver sections in the different experimental groups stained with Haematoxylin & Eosin. A-B: Liver sections in control group revealed radiating hepatic cords from central vein (CV) with interfering blood sinusoid (S). The hepatocytes appeared as polygonal cell having rounded vesicular basophilic nuclei (arrow) with acidophilic cytoplasm (curved arrow). Notice the portal area containing branch of the portal vein (PV) and a bile ductile (D). D-F: Liver sections from Etoposide group shows congestion and dilation of central (CV) and portal (PV) veins. The blood sinusoids (S) are also congested (angled arrow). The hepatocytes appear with deeply stained pyknotic nuclei (arrow) with vacuolated cytoplasm (curved arrow) and some hepatocytes exhibited vacuolar degeneration (arrow head). Extensive lymphocytic cellular infiltration (IF) and proliferation of bile duct (D) is detected. G-H: Liver sections from rats treated with Etoposide with rosemary group reveals a marked improvement as most of hepatocytes exhibit vesicular nuclei (arrow) with acidophilic cytoplasm (curved arrow) however some hepatocytes still have pyknotic nuclei (zigzag arrow). The hepatocyte appear radiating from central vein(CV) with interfering blood sinusoid (S). The portal area contains a branch of the portal vein (PV) and a bile ductile (D).

in their original site or far from the original tumor origin, and stop their rapid growth (Tousson et al., 2014). One of the side effects of chemotherapy is that it sometimes does not differentiate between normal cells and cancerous cells, so chemotherapy kills fast-growing cells in the body, whether they are normal cells or cancer cells, including blood cells and hair cells. Therefore, scientists have recently been interested in finding therapies that decrease the side effects of chemotherapy without altering its effectiveness or rising its toxicity (Basuony et al., 2015). The purpose of the present work is to study if rosemary extract can protect male albino rats from etoposide-induced liver and kidney damage.

Chemotherapy patients often have hepatotoxicity, which alters their liver function tests. Hepatotoxicity occurs in the form of loss of appetite, fatigue, nausea, pain in the

right upper quadrant, dark urine, and jaundice. Compared to control rats, etoposide therapy resulted in a significant increase in serum ALT, AST, and ALP, as well as a significant drop in albumen and total proteins, indicating liver injury. This finding is consistent with the findings of Nasr (2013), Al-Ameri (2017), Almakhatreh et al. (2019), who reported that etoposide administration significantly increased the levels of ALT, AST, and ALP, as well as a significant decrease in albumen and total proteins in the serum, all of which are consistent with liver toxicity.

These findings are consistent with those of Nasr (2013), Al-Ameri (2017), who found that Cisplatin treatment increased ALT, AST, and ALP levels while decreasing albumen and total proteins. Also, according to Jahovic et al. (2003), methotrexate-induced hepatotoxicity and nephrotoxicity in male rats, as well as enhanced free radical-associated liver function, leads to cell damage by binding to large cellular macromolecules, leads to cell damage. McDonald et al., 2003 published similar findings, stating that cyclophosphamide-induced hepatotoxicity in the liver of human. Higher levels of the AST and ALT enzymes in the serum indicate a lack of cell membrane function and cellular leakage in the liver (Gupta et al., 2009). The Estimating these enzymes is important in knowing the degree and type of damage to liver cells (Habtemariam, 2016).

One of the unusual side effects of chemotherapy is nephrotoxicity (Al-Ameri, 2017). Chemotherapy-induced nephrotoxicity leads to abnormal alteration of renal function tests in animal models and patients. Renal toxicity caused by chemotherapy leads to tubular or glomerular dysfunction or a combination of both (Basuony et al., 2015). In this study, a significant increase in serum creatinine, urea, potassium and chloride was found, while calcium ions decreased significantly in the etoposide group. The increase in serum creatinine, urea, chloride and potassium levels in etoposide-treated rats is an important marker of kidney dysfunction. This finding is in harmony with Al-Ameri (2017) who showed that etoposide caused electrolyte changes and nephrotoxicity in male rats. The finding of this study confirmed those of Basuony et al. (2015), who found that cisplatin causes nephrotoxicity in rats. In addition, the findings of this study matched those of Nasr (2013), who showed that high doses of etoposide, carboplatin, and ifosfamide can cause nephrotoxicity in humans.

Etoposide treatment leads to a lack of lipid standardized antioxidants, thus reducing antioxidants and increasing the formation of free radicals as well as the level of lipid hydroperoxide in the serum, which leads to oxidative stress. Peroxidation of lipids leads to damage to cellular lipid content, which causes damage to cells and this occurs by the interaction of lipids with free radicals, and this reaction leads to the production of products such as hydrogen peroxide. The increase of these highly reactive free radicals leads to the release of reactive oxygen species, causing harmful effects in various tissues of the body (McDonald et al., 2003). Reactive oxygen species (ROS) such as H_2O_2 can be generated after etoposide treatment (Namazi et al., 2015). the excess in production of H_2O_2 and O_2 by auto-oxidation to increase glucose and non-enzymatic glycoproteins may be the reason for decreased SOD and CAT activities (Aragno et al., 1997). This work reported that administration of rosemarry to rats for three times weekly improved the activities of SOD, GPX and CAT in rat's liver. One of the plants rich in various phytochemical derivatives such as polyphenols, flavonoids, and triterpenes is rosemary (Rašković et al., 2014). In large part, the protective properties of rosemary are a result of its antioxidant and radical scavenging properties, as well as its electron donation against different types of reactive nitrogen and oxygen species (Hasanein and Sharifi, 2017). Phytochemical examination of rosemary showed the presence of many active components such as flavonoids, polyphenols and diterpene which have strong antioxidant properties (Moreno et al., 2006). These phytochemicals are able to give electrons to the reactive radicals, and thus these reactive radicals become non-reactive and more stable, when these reactive radicals become more stable, they do not interact with biomolecules, like DNA, lipoproteins and polyunsaturated fatty acids (Rašković et al., 2014). Rosemary's protective effect may work by induction of detoxifying enzymes. Sometimes rosemary or one of its components is a cofactor in the production of endogenous antioxidants such as GST and quinine reductase (Singletary, 1996). All of this supports our discovery of the presence of enhanced antioxidants in liver tissue treated with rosemary extract. All of these mechanisms support our results showing the protective effect of rosemary extract versus toxicity of etoposide (Aragno et al., 1997).

In the present study serum level of T3 and T4 was significantly increased in etoposide-administered rats. This could be due to oxidative stress caused by etopside. Oxidative stress appears to be a key factor in the progression of inflammation. Thyroid hormones can protect the body by controlling antioxidant levels. There is a correlation between oxidative stress and hormonal imbalance. Thyroid hormones have an important role in the balance of antioxidants, and it has been found that there is a link between hyperthyroidism and hypothyroidism with oxidative stress in animals and humans (Kang and Hamasaki, 2003; Valko et al., 2007). Specifically, thyroid hormones assume significant parts in antioxidant variation, as exhibited in various in vitro and in vivo observes. Oxidative stress has been demonstrated to be related with both hyperthyroidism and hypothyroidism. Although, the mechanisms by which oxidative stress is created in these two clinical conditions are dissimilar: expanded ROS creation in hyperthyroidism and low low availability of antioxidants in hypothyroidism (Resch et al., 2002).

Measuring gene expression is important after drug exposure, as it gives information about gene regulation and biological processes after drug exposure, and also knows the response to these drugs. The transcriptomic analysis gives a specific pattern of genes that may indicate toxicity caused by drug, and gives more specific and accurate information from biomarkers as well as specific knowledge of the mechanism of toxicity induced by drug. Moreover, biomarkers usually detect the toxicity of traditional drugs when the damage has already occurred while changes in gene expression can occur immediately, and knowledge of these changes in gene expression allows for a more rapid and effective prediction of intestinal infection. Although gene expression production promises

to inform biomarker investigation (Bauer et al., 2016; Li et al., 2017; Matsuyama et al., 2019; Toma et al., 2017), disease progression and personalized medicine (Lowe et al., 2017; Dong and Chen, 2013), it is still clear that there are few studies that treat drug-induced responses to gene expression. Rosemary extract resulted in a significant reduction in hepatic activity as well as SOD and GPx gene expression. The activity of enzymatic and non-enzymatic antioxidant defences in the liver of etoposide-intoxicated rats was shown to be reduced, which was linked to etoposide-induced oxidative stress (Mahmoud, 2014). SOD and GPx are important antioxidants that protect the body from free radicals and ROS (Wei et al., 2011). As a result, (RE) appears to defend against etoposide by increasing the antioxidant defenses in the liver.

In the current study, the overexpression of the transcription factor Nrf2 may be directly linked to an increase in the production and activity of antioxidant defence enzymes by rosemary extract. Because it binds to cis-acting ARE and promotes antioxidant and defence gene production, Nrf2 is important in tissue protection from oxidants (Kensler et al., 2007). Under normal physiological conditions, Nrf2 is inactivated in the cytoplasm by forming a complex with its repressor Kelch-like ECH2 related protein (Keap 1) (Kang et al., 2004). Nrf2 splits from Keap 1, travels into the nucleus, binds to ARE, and induces SOD and GPx expression (Patel and Maru, 2008; Bardag-Gorce et al., 2011). Thus, overexpression of Nrf2 can lead to a reduction in responsive oxidants by increasing the expression of antioxidant defence enzymes, resulting in less cell damage. This study found a predicted decrease in Nrf2 mRNA and protein expression in the liver tissue of etoposide-treated rats, which was reversed by RE administration. The level of Nrf2 expression is strongly linked to the levels of SOD and GPx; nevertheless, the particular mechanism of RE-initiated Nrf2 overexpression requires more investigation.

The histopathological results displayed that, after treatment with etoposide, moderate atrophied vacuolar degeneration hepatocytes, some apoptotic cells with deep eosinophilic cytoplasm and small deeply stained pyknotic or fragmented nuclei, marked cellular infiltrations, and marked dilation or congestion in central and portal veins, as well as blood sinusoids, were observed. Bile duct proliferation was also discovered. Similar changes were recorded by other investigators (Ravindra et al., 2012; Tousson et al., 2019). The production of free radicals is largely responsible for the enormous degenerative alterations produced by etoposide. The protective effect of rosemary extract was also confirmed by histopathological examination. The majority of the etoposide-induced damage in liver tissue was significantly reduced after treatment with rosemary extract, indicating that they had the potential to scavenge free radicals. According to one of its active components, rosmarinic acid, rosemary is frequently used by herbalists and naturopaths for its therapeutic advantages on liver disease. This is similar to observations made by Al-Attar and Shawush (2015) who discovered that administering rosemary extract before or during thioacetamide therapy enhanced all biochemical indicators as well as the histological picture of the liver. Li et al. (2010) and Abdel-Wahhab et al. (2011) found that rosemary extract reduces CCl4-induced hepatotoxicity in rats by scavenging or preventing the production of free radicals produced during CCl4 metabolism. The bioactive components in rosemary may be responsible for reducing adverse effects of CCl4 through scavenging activity or antioxidant properties that decreased lipid peroxidation, stabilized reactive radicals, and protected cellular integrity.

5. Conclusions

The present study concluded that rosmarinus officinalis extract has a protective potential against etoposide-induced oxidative stress and toxicity in male albino rats. As a result, taking rosemary extract may help patients undergoing chemotherapy improve their general health.

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References

ABDEL-WAHHAB, K.G.E., EL-SHAMY, K.A., EL-BEIH, N.A.E., MORCY, F.A. and MANNAA, F.A.E., 2011. Protective effect of a natural herb (*Rosmarinus officinalis*) against hepatotoxicity in male albino rats. *Comunicata Scientiae*, vol. 282, no. 2, pp. 9-17.

AL-AMERI, A.S.H., 2017. Prevention of etoposide induced kidney toxicity, electrolytes, injury and KI67 alternations in male rats treated with star anise. *Journal of Bioscience and Applied Research*, vol. 3 no. 2, pp. 36-41. http://dx.doi.org/10.21608/jbaar.2017.124881.

AL-ATTAR, A.M. and SHAWUSH, N.A., 2015. Influence of olive and rosemary leaves extracts on chemically induced liver cirrhosis in male rats. *Saudi Journal of Biological Sciences*, vol. 22, no. 2, pp. 157-163. http://dx.doi.org/10.1016/j.sjbs.2014.08.005. PMid:25737646.

ALMAKHATREH, M., HAFEZ, E., TOUSSON, E. and MASOUD, A., 2019. Biochemical and molecular studies on the role of rosemary (Rosmarinus officinalis) extract in reducing liver and kidney toxicity due to etoposide in male rats. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, vol. 5, pp. 1-11. http://dx.doi.org/10.9734/ajrimps/2019/v7i430126.

AL-RASHEED, N.M., EL-MASRY, T.A., TOUSSON, E., HASSAN, H.M. and AL-GHADEER, A., 2017. Protective potential of grape seed proanthocyandins extract against Glivec (Imatinib mesylate) induced liver toxicity and oxidative stress in male rats. *Annual Research & Review in Biology*, vol. 20, no. 6, pp. 1-9. http://dx.doi.org/10.9734/ARRB/2017/37766.

ARAGNO, M., BRIGNARDELLO, E., TAMAGNO, E., GATTO, V., DANNI, O. and BOCCUZZI, G., 1997. Dehydroeppiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. *The Journal of Endocrinology*, vol. 155, no. 2, pp. 233-240. http://dx.doi.org/10.1677/joe.0.1550233. PMid:9415057.

BARDAG-GORCE, F., OLIVA, J., LIN, A., LI, J., FRENCH, B.A. and FRENCH, S.W., 2011. Proteasome inhibitor up regulates liver antioxidative enzymes in rat model of alcoholic liver disease.

Experimental and Molecular Pathology, vol. 90, no. 1, pp. 123-130. http://dx.doi.org/10.1016/j.yexmp.2010.10.013. PMid:21036165.

BARTOSZ, G., 2006. Use of spectroscopic probes for detection of reactive oxygen species. *Clinical Chemistry*, vol. 368, no. 1-2, pp. 53-76. http://dx.doi.org/10.1016/j.cca.2005.12.039. PMid:16483560.

BASUONY, M., HAFEZ, E., TOUSSON, E., MASSOUD, A., ELSOMKHRATY, S. and ELDAKAMAWY, S., 2015. Beneficial role of Panax ginseng root aqueous extract against cisplatin induced blood toxicity in rats. *American Journal of Biological Chemistry*, vol. 3, no. 1, pp. 1-7.

BAUER, M., GIAMARELLOS-BOURBOULIS, E.J., KORTGEN, A., MÖLLER, E., FELSMANN, K., CAVAILLON, J.M., GUNTINAS-LICHIUS, O., RUTSCHMANN, O., RURYK, A., KOHL, M., WLOTZKA, B., RUSSWURM, S., MARSHALL, J.C. and REINHART, K., 2016. A transcriptomic biomarker to quantify systemic inflammation in sepsis – a prospective multicenter phase II diagnostic study. *EBioMedicine*, vol. 6, pp. 114-125. http://dx.doi.org/10.1016/j.ebiom.2016.03.006. PMid:27211554.

BELFIELD, A. and GOLDBERG, D.M., 1971. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. *Enzyme*, vol. 12, no. 5, pp. 561-573. http://dx.doi.org/10.1159/000459586. PMid:5169852.

BRADFORD, M.M., 1976. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051.

CARNEIRO, J.L., NIXDORF, S.L., MANTOVANI, M.S., HERRERA, A.C.S.A., AOKI, M.N., AMARANTE, M.K., FABRIS, B.A., FUNGARO, M.H.P. and WATANABE, M.A.E., 2009. Plasma malondialdehyde levels and CXCR4 expression in peripheral blood cells of breast cancer patients. *Journal of Cancer Research and Clinical Oncology*, vol. 135, no. 8, pp. 997-1004. http://dx.doi.org/10.1007/s00432-008-0535-7. PMid:19125297.

COHEN, G., DEMBIEC, D. and MARCUS, J., 1970. Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, vol. 34, no. 1, pp. 30-38. http://dx.doi.org/10.1016/0003-2697(70)90083-7. PMid:5440916.

DONG, Z. and CHEN, Y., 2013. Transcriptomics: advances and approaches. *Science China. Life Sciences*, vol. 56, no. 10, pp. 960-967. http://dx.doi.org/10.1007/s11427-013-4557-2. PMid:24091688.

DORMAN, H.J.D., PELTOKETO, A., HILTUNEN, R. and TIKKANEN, M.J., 2003. Characterization of the antioxidant properties of deodourised aqueous extracts from selected Lamiaceae herbs. *Food Chemistry*, vol. 83, no. 2, pp. 255-262. http://dx.doi.org/10.1016/S0308-8146(03)00088-8.

FEARON, W.R., 1939. The carbamido diacetyl reaction: a test for citrulline. *Biochemestry Journal*, vol. 33, no. 6, pp. 902-907. http://dx.doi.org/10.1042/bj0330902. PMid:16746990.

GRANGER, D.L., TAINTOR, R.R., BOOCKVAR, K.S. and HIBBS JUNIOR, J.B., 1996. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. *Methods in Enzymology*, vol. 268, pp. 142-151. http://dx.doi.org/10.1016/S0076-6879(96)68016-1. PMid:8782580.

GUPTA, Y.K., BRIYAL, S. and SHARMA, M., 2009. Protective effect of curcumin against kainic acid induced seizures and oxidative stress in rats. *Indian Journal of Physiology and Pharmacology*, vol. 53, no. 1, pp. 39-46. PMid:19810575.

HABTEMARIAM, S., 2016. The therapeutic potential of rosemary (Rosmarinus officinalis) diterpenes for Alzheimer's disease. *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, p. 2680409. http://dx.doi.org/10.1155/2016/2680409. PMid:26941822.

HASANEIN, P. and SHARIFI, M., 2017. Effect of rosmarinic acid on acetaminophen-induced hepatotoxicity in male Wistar rats. *Pharmaceutical Biology*, vol. 55, no. 1, pp. 1809-1816. http://dx.doi.org/10.1080/13880209.2017.1331248. PMid:28545313.

HENERY, R.J., CANNON, D.C. and WINKELMAN, J.W., 1974. *Clinical chemistry: principles and techniques*, 2nd ed. New York: Harper and Row, vol. 2, pp. 422-424.

JAHOVIC, N., CEVIK, H., SEHIRLI, A.O., YEĞEN, B.C. and SENER, G., 2003. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. *Journal of Pineal Research*, vol. 34, no. 4, pp. 282-287. http://dx.doi.org/10.1034/j.1600-079X.2003.00043.x. PMid:12662351.

JAIN, A., SUBHAN, I. and JOSHI, M., 2009. Comparison of the point-of-care blood gas analyzer versus the laboratory auto-analyzer for the measurement of electrolytes. *International Journal of Emergency Medicine*, vol. 2, no. 2, pp. 117-120. http://dx.doi.org/10.1007/s12245-009-0091-1. PMid:20157454.

JARDIM-GONCALVES, R., GRILO, A., AGOSTINHO, C., LAMPATHAKI, F. and CHARALABIDIS, Y., 2013. Systematisation of interoperability body of knowledge: the foundation for enterprise interoperability as a science. *Enterprise Information Systems*, vol. 7, no. 1, pp. 7-32. http://dx.doi.org/10.1080/17517575.2012.684401.

KANCHANA, U., KISHORI, B., STREENIVASULA, P. and SREENIVASUL, R., 2013. Cisplatin and/or etoposide induces oxidative stree in testicular hepatic and kidney tissues in male albino mice. *Journal of Biolgy and Earth Sciences*, vol. 3, no. 2, pp. 249-254.

KANG, D. and HAMASAKI, N., 2003. Mitochondrial oxidative stress and mitochondrial DNA. *Clinical Chemistry and Laboratory Medicine*, vol. 41, no. 10, pp. 1281-1288. http://dx.doi.org/10.1515/CCLM.2003.195. PMid:14580153.

KANG, M.I., KOBAYASHI, A., WAKABAYASHI, N., KIM, S.G. and YAMAMOTO, M., 2004. Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 7, pp. 2046-2051. http://dx.doi.org/10.1073/pnas.0308347100. PMid:14764898.

KENSLER, T.W., WAKABAYASHI, N. and BISWAL, S., 2007. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annual Review of Pharmacology and Toxicology*, vol. 47, pp. 89-116. http://dx.doi.org/10.1146/annurev.pharmtox.46.120604.141046. PMid:16968214.

LEVESQUE, R., 2007. *SPSS programming and data management: a guide for SPSS and SAS users*. 4th ed. Chicago: SPSS Inc.

LI, G.-S., JIANG, W.-L., TIAN, J.-W., QU, G.-W., ZHU, H.-B. and FU, F.-H., 2010. In vitro and in vivo antifibrotic effects of rosmarinic acid on experimental liver fibrosis. *Phytomedicine*, vol. 17, no. 3-4, pp. 282-288. http://dx.doi.org/10.1016/j.phymed.2009.05.002. PMid:19524418.

LI, H.-H., CHEN, R., HYDUKE, D.R., WILLIAMS, A., FRÖTSCHL, R., ELLINGER-ZIEGELBAUER, H., O'LONE, R., YAUK, C.L., AUBRECHT, J. and FORNACE JUNIOR, A.J., 2017. Development and validation of a high-throughput transcriptomic biomarker to address 21st century genetic toxicology needs. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 51, pp. E10881-E10889. http://dx.doi.org/10.1073/pnas.1714109114. PMid:29203651.

LOWE, R., SHIRLEY, N., BLEACKLEY, M., DOLAN, S. and SHAFEE, T., 2017. Transcriptomics technologies. *PLoS Computational Biology*, vol. 13, no. 5, p. e1005457. http://dx.doi.org/10.1371/journal.pcbi.1005457. PMid:28545146.

MAHMOUD, A.M., 2014. Hesperidin protects against cyclophosphamide-induced hepatotoxicity by upregulation of PPARγ and abrogation of oxidative stress and inflammation. *Canadian Journal of Physiology and Pharmacology*, vol. 92, no. 9, pp. 717-724. http://dx.doi.org/10.1139/cjpp-2014-0204. PMid:25079140.

MARKLUND, S. and MARKLUND, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, vol. 47, no. 3, pp. 469-474. http://dx.doi.org/10.1111/j.1432-1033.1974.tb03714.x. PMid:4215654.

MATKOVICS, B., 1988. Determination of enzyme activity in lipid peroxidation and glutathione pathways. *Laboratoriumi Diagnosztika*, vol. 15, pp. 248-250.

MATSUYAMA, T., ISHIKAWA, T., TAKAHASHI, N., YAMADA, Y., YASUNO, M., KAWANO, T., UETAKE, H. and GOEL, A., 2019. Transcriptomic expression profiling identifies ITGBL1, an epithelial to mesenchymal transition (EMT)-associated gene, is a promising recurrence prediction biomarker in colorectal cancer. *Molecular Cancer*, vol. 18, p. 19. http://dx.doi.org/10.1186/s12943-019-0945-y. PMid:30717807.

MCDONALD, G.B., SLATTERY, J.T., BOUVIER, M.E., REN, S., BATCHELDER, A.L., KALHORN, T.F., SCHOCH, H.G., ANASETTI, C. and GOOLEY, T., 2003. Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. *Blood*, vol. 101, no. 5, pp. 2043-2048. http://dx.doi.org/10.1182/blood-2002-06-1860. PMid:12406916.

MENA, P., CIRLINI, M., TASSOTTI, M., HERRLINGER, K.A., DALL'ASTA, C. and RIO, D., 2016. Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (Rosmarinus officinalis L.) extract. *Molecules*, vol. 21, no. 11, p. 1576. http://dx.doi.org/10.3390/molecules21111576. PMid:27869784.

MONTECUCCO, A., ZANETTA, F. and BIAMONTI, G., 2015. Molecular mechanisms of etoposide. *Expermintal and Clinical Journal*, vol. 14, pp. 95-108. PMid:26600742.

MORENO, S., SCHEYER, T., ROMANO, C.S. and VOJNOV, A.A., 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research*, vol. 40, no. 2, pp. 223-231. http://dx.doi.org/10.1080/10715760500473834. PMid:16390832.

NAMAZI, H., KULISH, V.V. and WONG, A., 2015. Mathematical modelling and prediction of the effect of chemotherapy on cancer cells. *Scientific Reports*, vol. 5, no. 1, p. 13583. http://dx.doi.org/10.1038/srep13583. PMid:26316014.

NASR, A.Y., 2013. Morphological, biochemical, histological, and ultrastructural protective effects of misoprostol on cisplatin induced hepatotoxicity in adult male rats. *Saudi Medical Journal*, vol. 34, no. 12, pp. 1237-1247. PMid:24343463.

OYOUNI, A.A., SAGGU, S., TOUSSON, E. and REHMAN, H., 2018. Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity,modified PCNA and Bcl-2 expression attenuated by Ocimum *basilicum* L. in CD1 mice. *Toxicology Reports*, vol. 5, pp. 687-694. http://dx.doi.org/10.1016/j.toxrep.2018.06.003. PMid:30003047.

PATEL, R. and MARU, G., 2008. Polymeric black tea polyphenols induce phase II enzymes via Nrf2 in mouse liver and lungs. *Free Radical Biology & Medicine*, vol. 44, no. 11, pp. 1897-1911. http://dx.doi.org/10.1016/j.freeradbiomed.2008.02.006. PMid:18358244.

PREUSS, H.G., JARRELL, S.T., SCHECKENBACH, R., LIEBERMAN, S. and ANDERSON, R.A., 1998. Comparative effects of chromium, vanadium and gymnema sylvestre on sugar-induced blood pressure elevations in SHR. *Journal of the American College of Nutrition*, vol. 17, no. 2, pp. 116-123. http://dx.doi.org/10.1080/07315724.1998.10718736. PMid:9550454.

RAŠKOVIĆ, A., MILANOVIĆ, I., PAVLOVIĆ, N., ĆEBOVIĆ, T., VUKMIROVIĆ, S. and MIKOV, M., 2014. Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. *BMC Complementary and Alternative Medicine*, vol. 14, p. 225. http://dx.doi.org/10.1186/1472-6882-14-225. PMid:25002023.

RAVINDRA, P., KULKARNI, S.R., DHUME, C.Y. and BHIWGADE, D.A., 2012. Histopothological and biochemical studies of etoposoide treated liver of rat. *International Journal of Pharma and Bio Sciences*, vol. 3, no. 2, pp. 1-11.

RESCH, U., HELSEL, G., TATZBER, F. and SINZINGER, H., 2002. Antioxidant status in thyroid dysfunction. *Clinical Chemistry and Laboratory Medicine*, vol. 40, no. 11, pp. 1132-1134. http://dx.doi.org/10.1515/cclm.2002.198. PMid:12521231.

ROLINSKI, B., KÜSTER, H., UGELE, B., GRUBER, R. and HORN, K., 2001. Total bilirubin measurement by photometry on a blood gas analyzer: potential for use in neonatal testing at the point of care. *Clinical Chemistry*, vol. 47, no. 10, pp. 1845-1847. http://dx.doi.org/10.1093/clinchem/47.10.1845. PMid:11568098.

SAGGU, S., SAKERAN, M.I., ZIDAN, N., TOUSSON, E., MOHAN, A. and REHMAN, H., 2014. Ameliorating effect of chicory (Chichoriumintybus L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. *Food and Chemical Toxicology*, vol. 72, pp. 138-146. http://dx.doi.org/10.1016/j.fct.2014.06.029. PMid:25010453.

SALAMA, A.F., TOUSSON, E., IBRAHIM, W. and HUSSEIN, W.M., 2013. Biochemical and histopathological studies of the PTU induced hypothyroid rat kidney with reference to the ameliorating role of folic acid. *Toxicology and Industrial Health*, vol. 29, no. 7, pp. 600-608. http://dx.doi.org/10.1177/0748233711432577. PMid:22287619.

SCHUMANN, G. and KLAUKE, R., 2003. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. *Clinica Chimica Acta*, vol. 327, no. 1-2, pp. 69-79. http://dx.doi.org/10.1016/S0009-8981(02)00341-8. PMid:12482620.

SHIN, H.J., KWON, H.K., LEE, J.H., ANWAR, M.A. and CHOI, S., 2016. Etoposide induced cytotoxicity mediated by ROS and ERK in human kidney proximal tubule cells. *Scientific Reports*, vol. 6, no. 1, p. 34064. http://dx.doi.org/10.1038/srep34064. PMid:27666530.

SINGLETARY, K.W., 1996. Rosemary extract and carnosol stimulate rat liver glutathione-S-transferase and quinone reductase activities. *Cancer Letters*, vol. 100, no. 1-2, pp. 139-144. http://dx.doi.org/10.1016/0304-3835(95)04082-X. PMid:8620433.

TOMA, M., MAK, G.J., CHEN, V., HOLLANDER, Z., SHANNON, C.P., LAM, K.K., NG, R.T., TEBBUTT, S.J., WILSON-MCMANUS, J.E., IGNASZEWSKI, A., ANDERSON, T., DYCK, J.R.B., HOWLETT, J., EZEKOWITZ, J., MCMANUS, B.M. and OUDIT, G.Y., 2017. Differentiating heart failure phenotypes using sex-specific transcriptomic and proteomic biomarker panels. *ESC Heart Failure*, vol. 4, no. 3, pp. 301-311. http://dx.doi.org/10.1002/ehf2.12136. PMid:28772032.

TOUSSON, E., BAYOMY, M.F. and AHMED, A.A., 2018. Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. *Biomedicine and Pharmacotherapy*, vol. 98, pp. 769-774. http://dx.doi.org/10.1016/j.biopha.2018.01.025. PMid:29571245.

TOUSSON, E., HAFEZ, E., MASOUD, A. and HASSAN, A.A., 2014. Abrogation by curcumin on testicular toxicity induced by cisplatin in rats. *Journal of Cancer Research and Treatment*, vol. 2, no. 3, pp. 64-68.

TOUSSON, E., MASOUD, A., HAFEZ, E. and ALMAKHATREH, M., 2019. Protective role of rosemary extract against etoposide induced liver toxicity, injury and KI67 alterations in rats. *Journal of Biochemistry and Applied Research*, vol. 5, no. 1, pp. 1-7. http://dx.doi.org/10.21608/jbaar.2019.105884.

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M.T.D., MAZUR, M. and TELSER, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, vol. 39, no. 1, pp. 44-84. http://dx.doi.org/10.1016/j.biocel.2006.07.001. PMid:16978905.

WEI, X.J., HU, T.J., CHEN, J.R. and WEI, Y.Y., 2011. Inhibitory effect of carboxymethylpachymaran on cyclophosphamide-induced oxidative stress in mice. *International Journal of Biological Macromolecules*, vol. 49, no. 4, pp. 801-805. http://dx.doi.org/10.1016/j.ijbiomac.2011.07.017. PMid:21821063.

WHITFIELD, J.B., 2001. Gamma glutamyl transferase. *Critical Reviews in Clinical Laboratory Sciences*, vol. 38, no. 4, pp. 263-355. http://dx.doi.org/10.1080/20014091084227. PMid:11563810.

WOLFE, D., 2012. Tissue processing. In: S.K. SUVARNA, C. LAYTON and J. BANCROFT, eds. *Bancroft's theory and practice of histological techniques*. 7th ed. Amsterdam: Elsevier, pp. 105-123.

YUAN, J.-N., CHAO, Y., LEE, W.-P., LI, C.-P., LEE, R.-C., CHANG, F.-Y., YEN, S.-H., LEE, S.-D. and WHANG-PENG, J., 2008. Chemotherapy with etoposide, doxorubicin, cispkatin,5-fluorouracil and leucovorin for patients with advanced hepatocellular carcinoma. *Medical Oncology*, vol. 25, no. 2, pp. 201-206. http://dx.doi.org/10.1007/s12032-007-9013-3. PMid:18488159.

ZHANG, X., YUAN, X., SHI, H., WU, L., QIAN, H. and XU, W., 2015. Exosomes in cancer: small particle, big player. *Journal of Hematology & Oncology*, vol. 8, p. 83-13. http://dx.doi.org/10.1186/s13045-015-0181-x. PMid:26156517.