Biochemical and molecular studies on the role of rosemary (*Rosemarinus officinalis)* extract in reducing liver and kidney toxicity due to etoposide in male rats

ABSTRACT

Aims: Etoposide is chemotherapeutic drugs that inhibit topoisomerase II activity and long been used for treatment of human malignancies, where it is a semi-synthetic compound derived from the plant Podophyllum pelltatum. The current study was designed to investigate the possible protective effect of rosemary extract against Etoposide -induced changes in liver and kidney functions, and DNA damage in rats.
Materials and Methods: A total of 50 male Wistar albino rats were divided randomly into four groups (1st group was control; 2nd group was treated with rosemary, 3rd group was received etoposide, and 4th & 5th groups was co- and post treated groups respectively).
Results: The administration of Etoposide revealed a significant increase in serum ALT, AST, ALP, creatinine, urea, potassium ions, chloride ions, and DNA damage. In contrast; a significant decrease in albumen, total proteins, sodium ions, and calcium ions were when compared with control group. This increased in ALT, AST, ALP, creatinine, urea, potassium ions, chloride ions, and DNA damage was reduced after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4. Also, this decreased in albumen, total proteins, sodium ions, and calcium ions was increased after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4.
Conclusion: It could be concluded that rosemary has a promising role and it worth to be considered as a natural substance for protective the liver and kidney toxicity induced by etoposide chemotherapy.

Key words: Chemotherapy; Liver; Kidney; Rat; Rosemary; Etoposide.

1. INTRODUCTION

Today, there are many different kinds of chemotherapy that used for cancer treatments. It is therefore important to search for therapies which can reduce the side effects of anticancer treatments without altering their efficacy or increasing toxicity or damage in target organs [1-8]. Etoposide or VP-16 is the trade name for etoposide. Etopophos and toposar or etoposide phosphate are other names for etoposide. In some cases, health care professionals may use the trade name VP-16 or other names vepesid or etopophos or toposar or etoposide phosphate when referring to the generic drug name etoposide. Etoposide is chemotherapeutic drugs that inhibit topoisomerase II activity and long been used for

treatment of human malignancies, where it is a semi-synthetic compound derived from the plant Podophyllum pelltatum [9,10].

Etoposide is commonly used alone or with another anticancer agent for the treatment of Hodgkin's lymphoma and AIDS and sexual organ cancers as testicular, ovarian, uterine, bladder and prostate or for the treatment of other organs as lung and stomach cancer [10]. Although etoposide is effective in the treatment of different types of cancers, it causes the death of normal proliferating cells, including male germ cells [9].

Many plant extracts and their products have been shown to have significant antioxidant activity which may be an important property of medicinal plants associated with the treatment of several ill-fated diseases including liver toxicity [11-19].

Rosemary (*Rosemarinus officinalis*) is one of household herbs that contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, and the antioxidants carnosic acid and it used in traditional medicine to treat a variety of disorders [20-22]. Extracts of rosemary leaves contains flavonoids and phenols and it possess a variety of bioactivities *in vitro* including anti-tumor, antioxidant, antibacterial, antinociceptive, antidiabetic, antithrombotic, antiulcerogenic, antidiuretic and anti-inflammatory agents [10,22]. Therefore; the present study was conducted to examine the possible modifying effects of rosemary aqueous extract against the changes in the liver and kidney function and DNA damage, induced by etoposide in male rats.

2. MATERIAL AND METHODS

2.1. Animals

The experiment was performed on 20 male rats weighing 150±10g and of 10-12 weeks age. The rats were held in suitable plastic cages for one week before the experimental work for acclimation in animal house at Zoology Department, Faculty of Science, Tanta University, Egypt and maintained on a standard rodent diet and water available *ad libitum*. After one week of acclimation, rats were equally divided into two groups. Animal maintenance and treatments were conducted in accordance with the Faculty of Science, Tanta University guide for animal, as approved by Institutional Animal Care and Use Committee (IACUC-SCI-TU-0019).

2.2. Chemical

Rosemary extract: The rosemary extract containing 40% carnosic acid was purchased from Hunan Geneham Biomedical Technological Company of China, (RAP20-110401).

Eoposide (Vepesid): VEPESID 100 mg capsule, (soft capsule) from Bristol-Myers Squibb Pharmaceuticals limited.

2.3. Experimental groups

Rats were equally divided into four groups.

1st group: Control group included rats that not received any treatment.

2nd group: Rosemary group included rats received by oral gavages rosemary extract at a dose of (220 mg/kg b.w. /twice weekly) for six weeks [21].

3rd group: Etoposide group included rats that injected intraperitoneally with etoposide (1 mg /kg B.W/day) for six weeks [10].

4th group: Co-treated group included rats that injected by etoposide (1 mg/kg B.W. /day) for six weeks and received rosemary (220 mg /kg b.w. /twice weekly) orally for the same six weeks.

4th group: Post treated group included rats that injected by etoposide (1 mg/kg B.W. /day) for six weeks and then received rosemary (220 mg /kg b.w. /twice weekly) orally for another six weeks.

At the end of the experimental period, rats were fasted overnight; euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

Blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes for estimation of liver and kidney functions biomarkers [23]. Blood samples were incubated at room temperature for 10 minutes and left to clot then centrifuged at 3000 r.p.m for 15 min and the serum were collected, serum was separated and kept in clean stopper plastic vial at –80°C until the analysis of serum parameters.

2.4. Liver function Biomarker:

Alanine transaminase (ALT) and aspartate transaminase (AST) activities in serum were assayed by using commercial kit that was supplied by Humann (Germany) according to the method of Tousson et al. [24] and Bolkiny et al. [25] respectively while alkaline phosphatase (ALP) was estimated in the rat serum according to El-Moghazy et al. [11]. Serum albumin was estimated according to Basuony et al. [6] while serum total proteins level was estimated according to Tousson et al. [26].

2.5. Electrolytes and kidney functions Biomarker:

Serum urea and creatinine were determined in the rat sera according to Salama et al. [27] and Eldaim et al. [28] respectively. To measure the levels of serum electrolytes (Potassium, sodium, calcium and chloride ions) by using commercial kits (Sensa core electrolyte, India) according to El Atrash et al. [29].

2.6. Comet assay

One gram of crushed kidney tissue was transferred to 1 mL of ice-cold phosphate-buffered saline (PBS) and the assay was performed according to Eldaim et al. [28], for visualization of DNA damage, observations were carried out on GelRed-stained DNA using a 40× objective on a fluorescent microscope. Komet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpoo1, UK) linked to a CCD camera was used to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculates tail moment. Generally, 50–100 randomly selected cells are analyzed per sample.

2.7. Statistical Analysis

Data were expressed as mean values ± SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $p<0.01$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

3. RESULTS

3.1. Serum markers of liver damage

Data presented in Figure (1) showed that serum ALT, AST, ALP and total bilirubin levels were significantly ($P<0.05$) increase in treated rats with etoposide as compared to control group. In contrast; a significant ($P<0.05$) decrease in total protein and albumin levels in treated rats with etoposide as compared to control group (Figure 1). Treatment of rats with etoposide and rosemary (as in G4&G5) revealed a significant ($P<0.05$) decrease in ALT, AST, ALP and total bilirubin levels and a significant ($P<0.05$) increase in total protein and albumin levels when compared with treated rats with etoposide (Figure 1). Also; with lowest damage in G4

3.2. Serum markers of kidney damage

Data presented in Figure (2) showed that serum creatinine, urea, potassium and chloride ions levels were significantly ($P<0.05$) increase in treated rats with etoposide as compared to control group. In contrast; a significant ($P<0.05$) decrease in serum sodium and calcium ions

levels in treated rats with etoposide as compared to control group (Figure 2). Treatment of rats with etoposide and rosemary (as in G4&G5) revealed a significant (P<0.05) decrease in creatinine, urea, potassium and chloride ions levels and a significant (P<0.05) increase in sodium and calcium ions levels when compared with treated rats with etoposide (Figure 1).

3.3. DNA damage in liver tissues

A comet assay was performed to assess DNA damage in liver of rats after treatment by rosemary and/or etoposide as compared to normal control. The results of comet assay were shown in Figures (3) and Tables (1). Administration of etoposide (G3) led to significant increase in liver DNA damage ($P < 0.05$) that was indicated by increase in tail length, tail DNA% and tail moment as compared to normal control (G1) and rosemary (G2) groups (Table 1 & Figure 3). This increased liver DNA damage was reduced after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4. On the other hand, no significant difference in liver DNA damage (tail length) was observed between normal control (G1) and rosemary treated groups (G2).

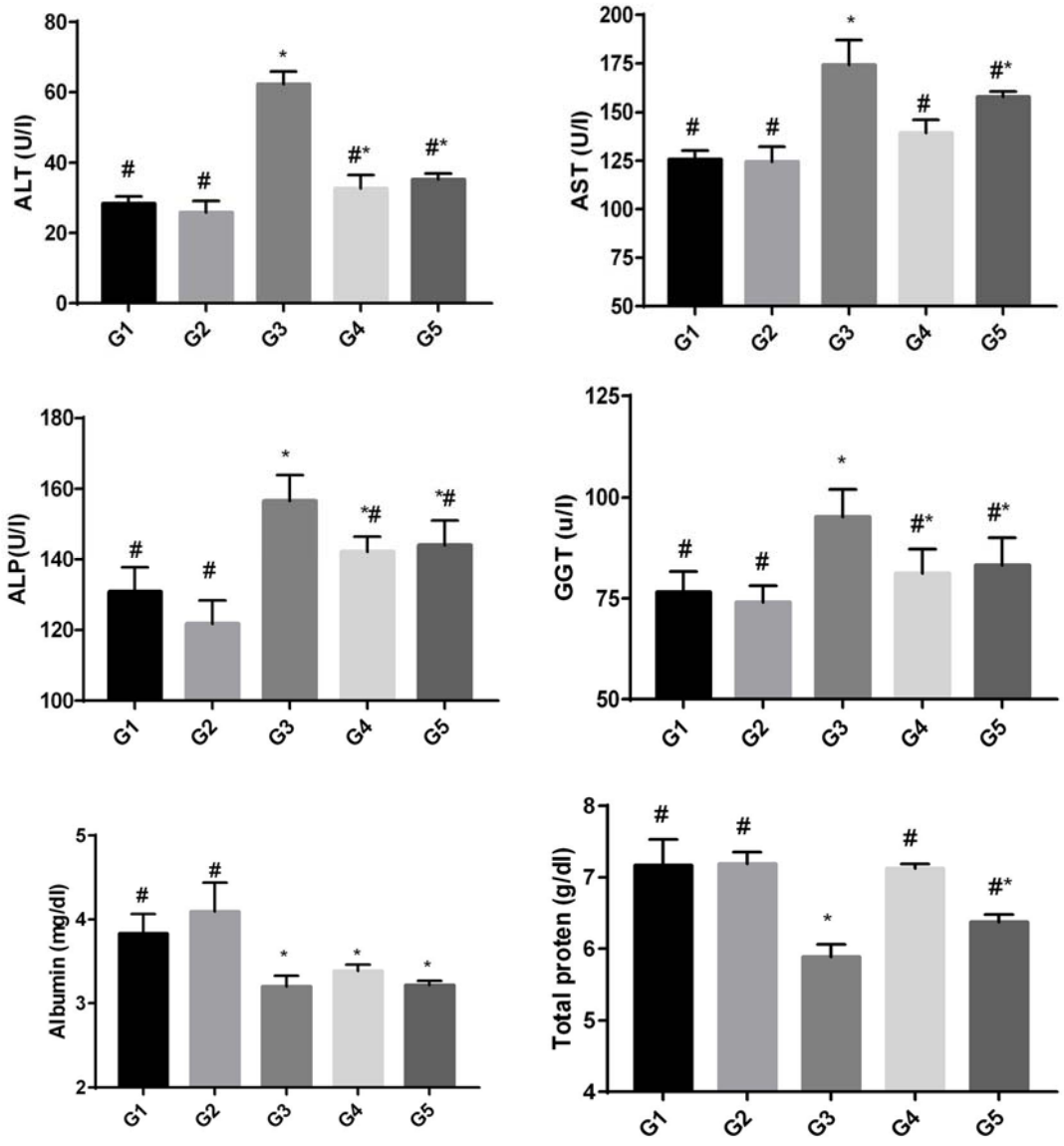

Figure 1: Changes in serum ALT, AST, ALP, GGT, total protein and albumin levels in different groups under study. Data are expressed as mean ± SE of 10 observations. *Significant difference from control group at *P<0.05*. #Significant difference from etoposide group at *P<0.05*.Where G1, control group; G2, rosemary group; G3, Etoposide group; G4, co-treated etoposide with rosemary group; G5, post-treated v

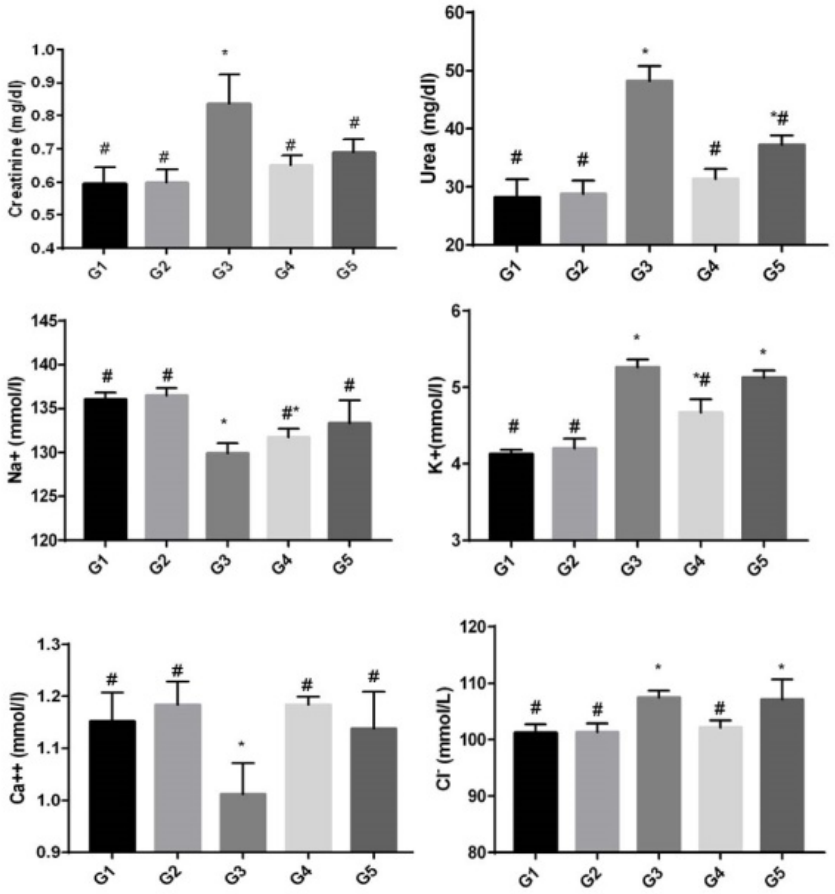

Figure 2: Changes in serum kidney functions (creatinine and urea) and electrolytes (sodium, potassium, calcium and chloride ions) levels in different groups under study. Data are expressed as mean ± SE of 10 observations. *Significant difference from control group at *P<0.05*. #Significant difference from etoposide group at *P<0.05*.Where G1, control group; G2, rosemary group; G3, Etoposide group; G4, co-treated etoposide with rosemary group; G5, post-treated etoposide with rosemary group.

Table 1: Comet assay parameters obtained by image analysis in liver cells of all groups after prevention experiment.

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
G1	4	96	1.79±0.35 [d]	1.68	3.04
G2	1.5	98.5	1.34±0.10 [d]	1.39	1.86
G3	19	81	6.55±0.34 [a]	5.05	33.08
G4	9	91	3.73±0.13 [c]	3.02	11.26
G5	12	88	4.62±0.21 [b]	3.70	17.09

Different superscript letters in the same column of tail length showed significance difference at P< 0.05. Where G1, control group; G2, rosemary group; G3, etoposide group; G4, co-treated etoposide with rosemary group; G5, post-treated etoposide with rosemary group.

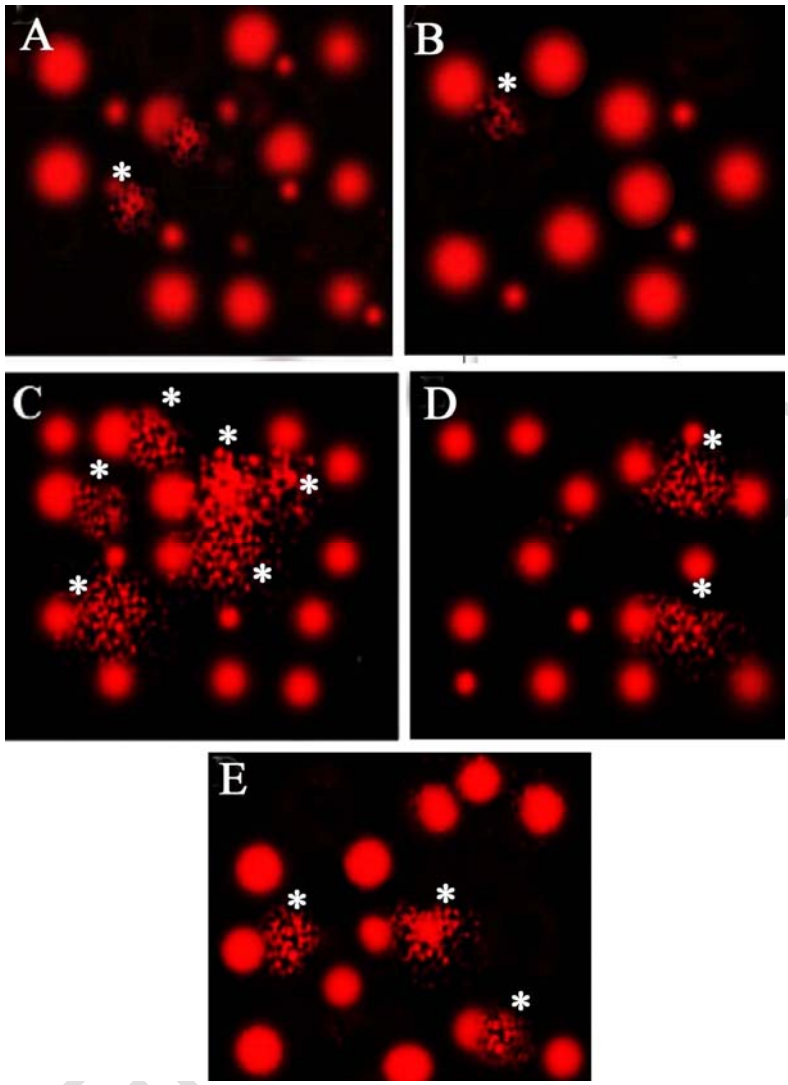

Figure 3: Photomicrographs representation of DNA damage in liver tissues, using comet assay, in normal control group (A), rosemary group (B), etoposide group (C), co-treated etoposide with rosemary group (D), and post-treated etoposide with rosemary group (E).

3.4. DNA damage in kidney tissues

A comet assay was performed to assess DNA damage in kidney of rats after treatment by rosemary and/or etoposide as compared to normal control. The results of comet assay were shown in Figures (4) and Tables (2). Administration of etoposide (G3) led to significant increase in kidney DNA damage ($P < 0.05$) that was indicated by increase in tail length, tail DNA% and tail moment as compared to normal control (G1) and rosemary (G2) groups (Table 2 & Figure 4). This increased kidney DNA damage was reduced after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4. On the other hand, no significant difference in kidney DNA damage (tail length) was observed between normal control (G1) and rosemary treated groups (G2).

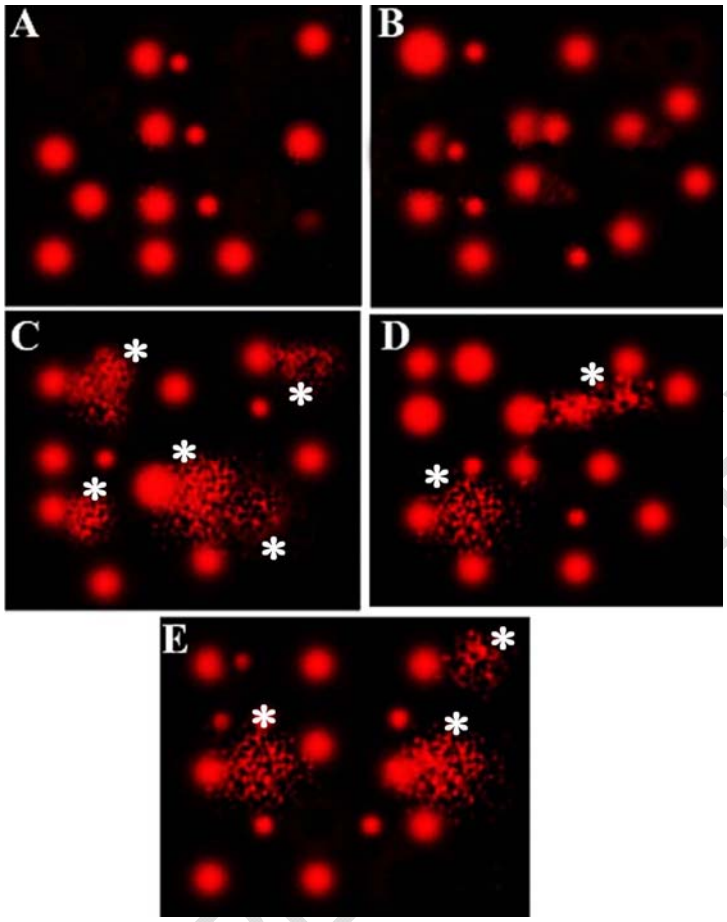

Figure 4: Photomicrographs representation of DNA damage in kidney tissues, using comet assay, in normal control group (A), rosemary control group (B), positive control group (C), co-treated etoposide with rosemary group (D), and post treated etoposide with rosemary group (E).

Table 2: Comet assay parameters obtained by image analysis in cells of all groups after treatment experiment.

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
G1	1.5	98.5	1.36±0.11 ^{d}	1.46	1.99
G2	3	97	1.48±0.12 ^{d}	1.60	2.37
G3	16	84	5.70±0.35 ^{a}	4.71	26.85
G4	7	93	3.11±0.17 ^{c}	2.28	7.09
G5	11	89	4.47±0.12 ^{b}	3.51	15.69

Different superscript letters in the same column of tail length showed significance difference at P< 0.05. Where G1, control group; G2, rosemary group; G3, etoposide group; G4, co-treated etoposide with rosemary group; G5, post-treated etoposide with rosemary group.

4. DISCUSSION

Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor [6]. However, it does not distinguish between a cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including, hair and blood cells. The current study aimed to study the protective and ameliorating effects of rosemary extract against liver toxicity induced by etoposide in male albino rats.

Chemotherapy-induced hepatotoxicity is a common cause of abnormal liver function test in patients, this hepatotoxicity are usually begins with vague clinical symptoms such as fatigue, anorexia, nausea, dark urine, right upper quadrant discomfort and jaundice. In the current study; a significant increase in serum ALT, AST, ALP and a significant decrease albumen and total proteins indicated the liver toxicity were detected after the treatments of rats with etoposide as compared with control. This result is in harmony with Abouzeinab [30]; Nasr [31]; Abdel-Wahhab et al. [32] and Basuony et al. [6] who reported that, Cisplatin administration induced an increase in ALT, AST, ALP and decrease albumen and total proteins. Also; this current result is in harmony with Tousson et al. [3,4] who reported that; methotrexate-induced hepatic and renal toxicity in male rats and the increased in liver function associated with free radicals trigger cell damage through binding to cellular macromolecules. Similar findings were reported by Juma [33] and McDonald et al. [34] who reported that; cyclophosphamide -Induced hepatotoxicity in human liver. Elevated levels of serum ALT and AST enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes in the liver [12,24]. The estimation of these enzymes in the serum is a useful quantitative marker for the extent and type of hepatocellular damage [7,8,35].

Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover [27,36,37]. Creatinine is the product of muscle creatine catabolism. Both are relatively small molecules (60 and 113 daltons, respectively) that distribute throughout total body water. The rationale for the use of creatinine or urea measurement to assess renal function is that plasma/serum levels of both reflect glomerular filtration rate (GFR), the parameter that defines kidney function for the clinician. Irrespective of its cause, kidney disease is associated with decrease in GFR, and the severity of kidney disease correlates closely but inversely with GFR [38].

Chemotherapy-induced renal toxicity is a common cause of abnormal kidney function test in patients and in animal models. Renal injury may follow treatment with anticancer drugs and lead to glomerular, tubular dysfunctions, or any combination of these [39]. Nephrotoxicity is an unusual side effect of chemotherapy in general. Most chemotherapy drugs target pathways that are essential to dividing cells. In the current study; serum creatinine, urea, potassium and chloride ions levels were significantly (P<0.05) increase in treated rats with etoposide as compared to control group. In contrast; a significant (P<0.05) decrease in serum sodium and calcium ions levels were detected in treated rats with etoposide as compared to control group. Mechanisms of anticancer drug-induced renal disorders generally include a varying degree of prerenal hypoperfusion, intrinsic renal damage, renal tubular obstruction, and damage to the microvascular structure of the kidneys [40]. Our result is agree with Tousson et al. [1] who find that MTX increased urea and creatinine activities which induced renal toxicity. Our result is agreed with Basuony et al. [6] who reported that; Cisplatin induced renal toxicity in rats. On the other hand, our results are disagreement with Cetiner et al. [41]. Our result is agreed with Beyer et al. [42] who reported that; High-dose carboplatin, etoposide and ifosfamide induced renal toxicity in human. Also; our result is agreed with Al-Ameri [43] who reported Etoposide induced kidney toxicity, electrolytes changes and injury. Chemotherapy-induced nephrotoxicity is a major cause of morbidity and mortality among cancer patients. Therefore, assessing baseline renal function before initiation of therapy and during therapy, adjusting drug dosages, avoiding nephrotoxic

drug combinations, and correcting the extracellular fluid volume depletion is essential in the cancer patients [44].

Treatment of rats with etoposide and rosemary revealed a significant decrease in creatinine, urea, potassium and chloride ions levels and a significant (P<0.05) increase in sodium and calcium ions levels when compared with treated rats with etoposide indicated that rosemary has renal protective against chemotherapy. The topoisomerase II inhibitor etoposide is an antineoplastic drug that has been widely used to couple DNA damage to apoptosis [45]. Topoisomerase II is a nuclear enzyme that functions during both DNA replication and transcription [46]. Topoisomerase II prevents “knots” from forming in DNA by allowing the passage of an intact segment of the helical DNA through a transient double strand break [47]. Topoisomerase II inhibitors such as etoposide stabilize the complex formed by topoisomerase II and the 5′-cleaved ends of the DNA, thus forming stable (nonrepairable) protein-linked DNA double strand breaks [47]. Cells are apparently able to recognize such DNA damage and, in turn, to eliminate the injured cells by apoptosis. In the current study, treatment of rats with etoposide led to significant increase in liver and kidney DNA damage ($P < 0.05$) that was indicated by increase in tail length, tail DNA% and tail moment as compared to normal control and rosemary groups. This increased kidney DNA damage was reduced after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4. Our results agree with Tousson et al. [10] who reported that; Etoposide induced DNA damage in testicular tissues.

5. CONCLUSION

Our recommendation is etoposide treatments induced changes in liver and kidney functions and DNA damage. Physicians should be aware of etoposide a differential diagnosis for hepatic and renal with an unknown etiology. Rosemary has a promising role and it worth to be considered as a natural substance for protective the liver and kidney toxicity induced by etoposide chemotherapy.

Conflict of interests

The authors declare no conflict of interest.

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