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Therapeutic Role of Rosemary Extract against Vepesid Induced Kidney Injury, Proliferation and Apoptosis in Male Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TFM and ET managed the analyses of the study. Author ET managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Vepesid (Etoposide) is a chemotherapeutic agent used in many types of tumors, but use it in the treatment is limited because of its toxicity in normal organ tissues. However, rosemary (*Rosmarinus officinalis*) aqueous extract has shown a protective effect against cancer and another disease.
Aims: In the current study, the protective effects of rosemary on vepesid-induced renal toxicity, injury, proliferation, and apoptosis in male rats were investigated.
Methods: Fifty male Wistar rats were divided into five equal groups: first group, control (G1); second group, rosemary (G2); third group, vepesid (G4); fourth group, co-treated rosemary plus vepesid (G4); fifth group, post-treated vepesid with rosemary (G5).
Results: Serum levels of urea, creatinine, potassium ions, and chloride ions significantly increased; while sodium ions and calcium were significantly decreased in vepesid group when compared with the control group. Besides, the renal histological structure showed marked degeneration and cellular infiltration in renal structure cells. Immunohistochemical investigations in kidney sections showed a

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moderate positive reaction for Ki67-ir immunoreactivity; while strong reactions for p53 protein in vepsid group when compared with the control group. In contrast, the treatment rats with rosemary extract in the co-treated and post-treated groups restored normal levels of kidney function parameters; it also improved the histological and immunohistochemical examinations in the kidney section when compared to vepsid group.
Conclusion: Rosemary extract has a renal potential protective effect against vepsid induced renal toxicity and injury.

Keywords: Rosemary; vepesid; kidney; rats; proliferation; apoptotic.

1. INTRODUCTION

Cancer is the most awful disease found among people and the largest single cause of death in human. Chemotherapy drugs cannot distinguish between the cancerous cells and the normal cells, which leads to side effects [1-7]. Although chemotherapy is one of the most effective methods for the treatment of cancer, it is often associated with several short and long-term toxicities and could lead to several toxic effects and clinically significant implications [8-10]. Vepesid is chemotherapeutic drugs that inhibit topoisomerase II activity and long been used for the treatment of human malignancies, where it is a semi-synthetic compound derived from the plant *Podophyllum peltatum* [11,12].Vepesid 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. Vepesidis commonly used alone or in combination with other anticancer agents in the treatment of lung or stomach cancer, Hodgkin's lymphoma, and AID's. Although etoposide is effective in the treatment of different types of cancers, it causes the death of normal proliferating cells, including male germ cells. 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 [13-16]. Medicinal plants are good sources of exogenous antioxidants which might be considered as the new alternative approach to ameliorate pathological alterations in oxidative stress-related pathology [17-20].

Rosemary (*Rosemarinus officinalis*) is one of the household herbs that contains several phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, and the antioxidants carnosic acid [21]. Extracts of rosemary leaves possess a variety of bioactivities *in vitro* including antioxidant, antibacterial, anti-tumor, antinociceptive, antiulcerogenic, antidiuretic, antidiabetic, anti-inflammatory, and antithrombotic agents [12,22]. Therefore; the present study was conducted to examine the possible modifying effects of rosemary aqueous extract against kidney toxicity, injury, Ki67, and P53 alterations induced by vepesid in male rats.

2. MATERIALS AND METHODS

2.1 Animals

The experiment was performed on 50 male rats weighing 250±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 an 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 5 groups. Animal maintenance and treatments were conducted by the Faculty of Science, Tanta University guide for the animal, as approved by the Institutional Animal Care and Use Committee (IACUC-SCI-TU-0019).

2.2 Chemical

2.2.1 Rosemary extract

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

2.2.2 Vepesid

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

2.3 Experimental Groups

Rats were equally divided into five 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.

3rd group: Vepesid group included rats that injected intraperitoneally with vepesid (1 mg /kg B.W/day) for six weeks.

4th group: Co-treated group included rats that injected by vepesid (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.

5th group: Post treated group included rats that injected by vepesid (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 have fasted overnight; euthanized with intraperitoneal injection with sodium pentobarbital, and subjected to a complete necropsy [23]. Blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes for the estimation of kidney function biomarkers. 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 was collected, serum was separated, and kept in clean stopper plastic vial at –80°C until the analysis.

2.4 Electrolytes and Kidney Functions Biomarkers

Serum urea and creatinine were determined in the mouse sera according to Salama et al. [24] and Mutar et al. [25] respectively. The method offered by Eldaim et al. [16] was used to measure the levels of serum electrolytes (Potassium, sodium, and calcium ions) using commercial kits (Sensa core electrolyte, India).

2.5 Histopathological Examination

Fixed kidney in 10% buffer neutral formalin was prepared for paraffin sectioning and subjected to histopathological examination using hematoxylin and eosin stains according to [5,26].

2.6 Immunohistochemical Detection of Apoptotic P53 Immunore activities

Sections were incubated overnight at 4°C after the application of the appropriate primary antibody. Sections incubated with anti-rabbit p53 monoclonal antibody for P53 expression (dilution 1:80; DAKO Japan Co, Ltd, Tokyo, Japan) according to Toussonet al. [27].

2.7 Immunohistochemical Detection of KI67 Immunorea ctivities

The expression of Ki67 immunoreactivity (Ki67-ir) detected using the avidin Biotin Complex (ABC) method Tousson et al. [3]. The sections were incubated with anti-mouse Ki67 monoclonal antibody (dilution 1:50, DAKO Japan Co, Ltd, Tokyo, Japan) for 1-2 h at room temperature according to Al-Rasheed et al. [28].

2.8 Statistical Analysis

Data were reported as mean values ± SE and one way ANOVA was used to detect significant differences between treatment groups. For biochemical results, the criterion for statistical significance was set at $p<0.01$.

3. RESULTS

3.1 Changes in Renal Functions Parameters

Data presented in (Tables 1 and2) showed that serum creatinine, urea, potassium, and chloride ions levels were significantly ($P < 0.05$) increase in treated rats with vepesid as compared to the control group. In contrast; a significant ($P < 0.05$) decrease in serum sodium and calcium ions levels in treated rats with vepesid as compared to the control group (Tables 1 and 2). Treatment of rats with vepesid and rosemary (as in G4&G5) revealed a significant ($P < 0.05$) decrease in creatinine, urea, potassium, and chloride ions levels; while a significant ($P < 0.05$) increase in sodium and calcium ions levels when compared with treated rats with vepesid group (Tables 1 and 2).

Table 1. Changes in serum kidney capacities (Creatinine and Urea) exercises in various gatherings under examination

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Control (G1)	$0.596^{\#}$ ± 0.022	$28.2^{\#}$ ± 1.393
Rosemary (G2)	$0.60^{\#}$ ± 0.017	$28.8^{\#}$ ± 1.020
Vepesid (G3)	0.838* ± 0.038	48.2* ± 1.158
Co-treated (G4)	$0.65^{\#}$ ± 0.014	$31.4^{\#}$ ± 0.748
Post-treated (G5)	$0.69^{\#}$ ± 0.018	$37.2^{*\#}$ ± 0.735

*Information is communicated as a mean ± SE of 10 perceptions. *Significant distinction from control bunch at P>0.01. #Significant distinction from vepsid bunch at P>0.01.Where G1, control gathering; G2, rosemary gathering; G3, Vepsid gathering; G4, co-treated vepsid with rosemary gathering; G5, post-treatedvepsid with rosemary gathering*

Table 2.Changes in serum kidney capacities (Na+, Cl-, Ca++and K+) exercises in various gatherings under examination

Groups	Na^{+}(mmol/l)	Cl^{-} (mmol/l)	Ca^{++}(mmol/l)	K^{+}(mmol/l)
Control (G1)	$136.1^{\#}$ ± 0.337	101.2 ± 0.675	$1.152^{\#}$ ± 0.025	$4.128^{\#}$ ± 0.026
Rosemary (G2)	$136.5^{\#}$ ± 0.373	101.3 ± 0.686	$1.184^{\#}$ ± 0.020	$4.2^{\#}$ ± 0.058
Vepesid (G3)	129.9* ± 0.530	107.4 ± 0.565	1.042* ± 0.036	5.26* ± 0.048
Co-treated (G4)	$131.7^{*\#}$ ± 0.443	102.1 ± 0.588	$1.184^{\#}$ ± 0.007	$4.672^{*\#}$ ± 0.077
Post-treated (G5)	$133.3^{\#}$ ± 1.182	107.1 ± 1.599	$1.138^{\#}$ ± 0.032	5.13* ± 0.040

*Information is communicated as a mean ± SE of 10 perceptions. *Significant distinction from control bunch at P>0.01. #Significant distinction from vepsid bunch at P>0.01.Where G1, control gathering; G2, rosemary gathering; G3, vepsid gathering; G4, co-treated vepsid with rosemary gathering; G5, post-treated Vepsid with rosemary gathering*

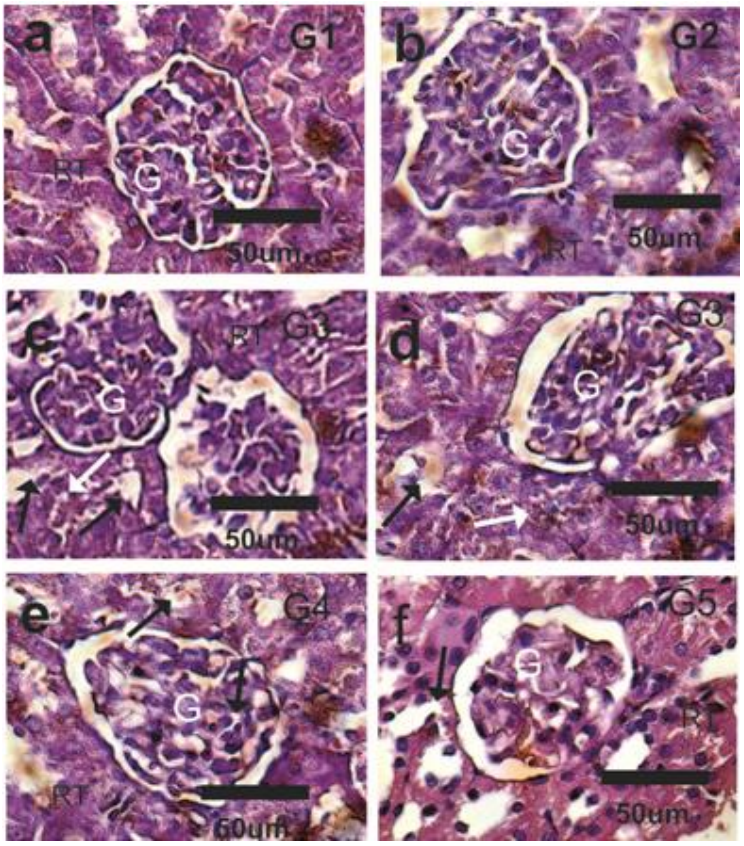

Fig. 1A-1F. Photomicrographs of rat kidney sections in all groups. A&B: Normal histological structure of kidney with glomeruli, (G) and renal tubules (RT) in the control.and in rosemary groups. C&D: Kidney sections in treated rat with Vepsid group. exhibited marked, glomerular injury, moderate, glomeruli atrophy (black arrows), necrosis (white arrows) moderate lymphocytic infiltration. E: Kidney sections in co-treated Vepsid with rosemary group exhibited mild glomeruli atrophy (arrows) and cellular infiltration in renal tubular cells. F: Kidney sections in post-treated vepsid with rosemary group exhibited moderate cellular infiltration in renal tubular cells (arrows)

3.2 Kidney Histopathology

Regarding the histopathological examination of the kidney from the rat's control and in the rosemary, groups showed normal renal cortex, and medulla, with normal histological features i.e. normal structure, were detected for glomeruli, and the Bowman's capsule with normal space between the glomeruli, and Bowman's capsule. The parietal layer of its renal capsule is composed of simple squamous epithelium. The renal corpuscles, of glomeruli, are surrounded by proximal, and distal, convoluted tubules (Fig. 1A and 1B).

Kidney sections in treated rats with chemotherapist drug vepsidgroup revealed marked, glomerular injury, moderate, glomeruli atrophy, moderate lymphocytic infiltration in addition to; degeneration in the renal tubular epithelial cells (Fig. 1C and1D). Kidney sections of co-treated vepsid with rosemary revealed good improvement in the kidney structure ad a mild glomeruli atrophy and cellular infiltration in renal tubular cells (Fig.1E). While kidney sections of post-treated vepsid with rosemary revealed mild improvement in the kidney structure with moderate cellular infiltration in renal tubular cells (Fig. 1F).

3.3 Ki67 Immunohistochemical Changes in the Kidney

The detection of immunore activity (Ki67-ir) in the rat kidney sections in the different groups was revealed in Fig. 2A-2F. Kidney sections in control and rosemary groups show a moderate positive reaction for Ki67-ir in both glomeruli and renal tubules nuclei (Fig. 2A and 2B). In contrast, mild positive reactions were detected for Ki67-ir in the kidney sections in vepsidtreated rats group (Fig. 2C and 2D). While a moderate positive reaction for Ki67-ir were observed in kidney sections of co-and post-treated vepsidwith rosemary (Fig. 2E and 2F).

3.4 P53 Immunohistochemical Changes in the Kidney

The detection of apoptotic P53 immunoreactivity (P53-ir) in the rat kidney sections in the different groups was revealed in Fig. 3A-3F. Kidney sections in control and rosemary groups show a negative to faint positive reaction for P53-ir in both glomeruli and renal tubules nuclei (Fig. 3A and 3B). In contrast moderate to strong positive reactions were detected for P53-ir in the kidney sections in vepsid treated rats group (Fig. 3C and 3D). In contrast, mild and moderate positive reactions for P53-ir were observed in kidney sections of co- and post-treated vepsid with rosemary respectively (Fig. 3E and 3F).

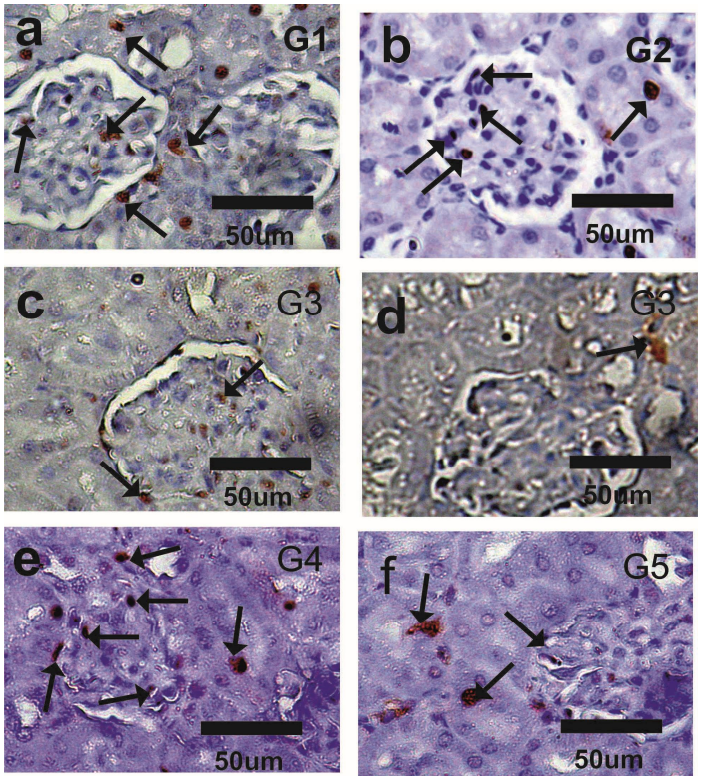

Fig. 2A-2F. Photomicrographs of rat kidney sections stained by Ki67 expressions. A&b: Moderate positive affinity for Ki67 in control (G1) group and rosemary (G2) groups. C&D: Faint positive affinity for Ki67 in kidney in treated rats with vepsid (G3). E: Moderate positive affinity for Ki67 in co-treated vepsid with rosemary. F: Mild positive affinity for Ki67 in post-treated vepsid with rosemary

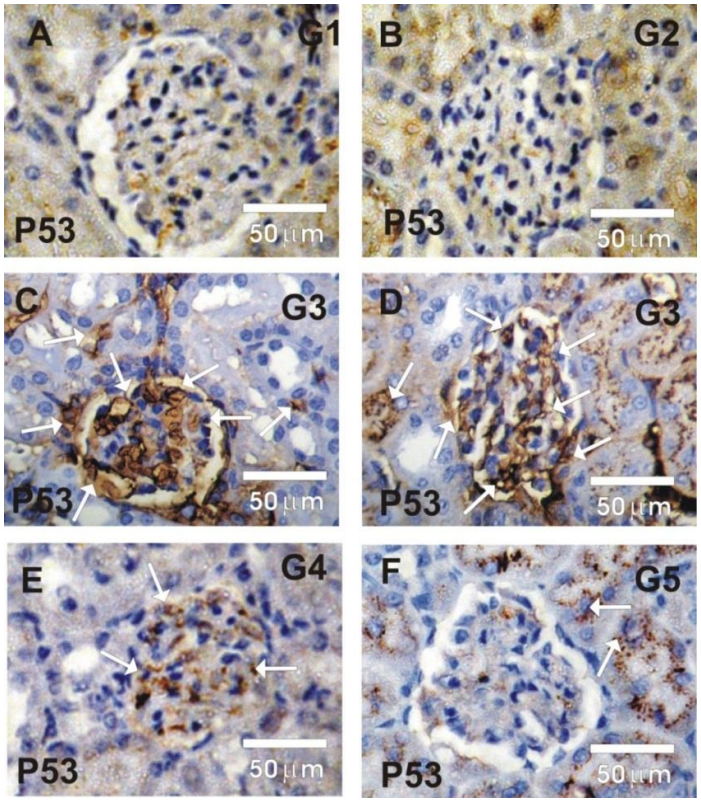

Fig. 3A-3F. Photomicrographs of rat kidney sections stained by P53 expressions. C&D: Strong positive affinity for P53 in the kidney in treated rats with vepsid (G3). D: Moderate positive affinity for P53 in co-treated vepsid with rosemary. F: Mild positive affinity for P53 in post-treated Vepsid with rosemary

4. DISCUSSION

This study conducts a biochemical and histopathological investigation into whether rosemary extract has a protective and ameliorated effect on vepesid - induced renal toxicity in male rats. 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 [29].

The kidney is an important targeted organ for xenobiotic compounds that produce renal toxicity including tubular cells and glomerulus [30,31]. Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover [31,32]. 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 a decrease in GFR, and the severity of kidney disease correlates closely but inversely with GFR [33].

Chemotherapy-induced renal toxicity is a common cause of abnormal kidney function tests in patients and animal models. Renal injury may follow treatment with anticancer drugs and lead to glomerular, tubular dysfunctions, or any combination of these [1,2,34]. Nephrotoxicity is an unusual side effect of chemotherapy in general. Most chemotherapy drugs target pathways that are essential to dividing cells [7,11].

Our study revealed that there are no symptoms of morbidity or mortality reported after oral administration of rosemary extracts in doses up to 100 mg/kg in rats suggesting that rosemary extracts were safe to be used and are nontoxic. In the current study, a significant elevation in serum urea, creatinine, potassium, and chloride while calcium ions were significantly decreased in vepesid group. The elevation in serum urea, creatinine, chloride and potassium levels in vepesid -treated rats is considered as a significant marker of renal dysfunction. Our result is agreed with Basuony et al. [7] who reported that; Cisplatin-induced renal toxicity in rats. Our result is agreed with Beyer et al. [35] who reported that; high-dose carboplatin, etoposide, and ifosfamide induced renal toxicity in human. Also; our result is agreed with Al-Ameri [36] who reported Etoposide induced kidney toxicity, electrolyte changes, and injury. Our result is agreed with Basuony et al. and Al-Ameri, [7,36] who reported that; Cisplatin-induced renal toxicity in rats also with Tousson et al. [1] who find that MTX increased urea and creatinine activities which induced renal toxicity. Treatment of rats with vepesid and rosemary revealed a significant decrease in creatinine, urea, potassium and chloride ions levels and a significant (P<0.01) increase in sodium and calcium ions levels when compared with treated rats with vepesid indicated that rosemary has renal protective against chemotherapy.

Antibodies to the cell-cycle-associated Ki-67 protein have been widely used for more than a decade as markers of proliferative cells. Ki-67 is a monoclonal antibody that is associated with cell proliferation and was first described by Duchrow et al. [37]. The presence of Ki-67 in all phases of cell division except G0 makes it an excellent marker for determining cell growth in target cells, especially in cancer cells [38]. The Ki-67 staining and tubule histology suggest a substantial diminution in tubule degeneration in mice and suppression of macrophages during their repair phase [39]. In the current study; a significant decrease in KI67 expression in kidney sections after treatment with vepesid when compared with control. In contrast, co-treatment vepesid with rosemary increases the depletion of Ki67-irin the kidney. Our histopathological and immune-histochemical results showed that the treatment of rat kidneys with aqueous extract of rosemary showed moderate to a good degree of improvement in the Malpighian corpuscles and renal tubules in kidney sections in vepesid group.

The p53 tumor suppressor gene has a role in reduced tumor growth through fight or arresting the cancer cells. The p53 protein induced cellular response when the occurrence of cancer and this response depends on the cell type and cell environment [40]. When damage occurs inside the cellular structure and DNA in the tissues, the activity of protein p53 will increase that leading to programmed cell death and DNA repair [41]. Moreover, Ikitimur-Armutak et al. [42] informed that carcinoma types with cytoplasmic confinement of p53 induced by chemotherapy or radiotherapy are less reacting to genotoxic stress; however, cytoplasmic accumulation of p53 is an autonomous unfavorable-prediction factor in a tumor.

Our results exposed that; strong positive reaction for apoptotic P53 was detected in renal sections of the vepesidgroup when compared with the normal control group. Our results agree with Tousson et al. [11] who reported that vepes id-induced a significant increase in P53 immunore activity. Our results exposed that; strong positive reaction for apoptotic P53 was detected in renal sections of the vepesid group when compared with the normal control group. Our results agree with Tousson et al. [11] who reported that vepesid induced a significant increase in P53 protein; while mild positive reactions were detected for Ki67-ir immunoreactivity in the male rats.

Numerous studies have indicated the protective and medical role of rosemary in many organs; however, these results agree with Tousson et al. [43] reported that rosemary improved the DNA damage, cellular injury, immunore activity (P53 and KI67) changes which induced by etoposide in rat testes. Also, Almakhatreh et al. [12] found that rosemary extract has an ameliorative role against vepesid-induced hepato-nephrotoxicity and DNA damage in male rats. Therefore, many natural plants have protective and medicinal properties against many diseases and toxins [44,45]. In the same context, Rasha and Abdella [46] reported that rosemary leaves aqueous extract has improving role on doxorubicin-induced apoptosis, oxidative stress, and histological injury in mice.

5. CONCLUSION

The significant restoration of all of the above biochemical and histopathological parameters towards normal values upon rosemary extract treatment tested in the present study indicates

the protection of vital organs such as the kidney from damage induced by vepesid. Hence, the present study confirms the potent renal protective and antioxidant nature of active phenolic compounds in rosemary extract; the strong antitumor activity observed in this model may be due to the antioxidant nature of the extract. Hence, it will be of great interest to isolate the active constituents of rosemary extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Male rat's ethics committee approval has been collected and preserved by the author.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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