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Prevention of Etoposide induced kidney toxicity, electrolytes, injury and KI67 alternations in male rats treated with star anise

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Abstract

Star anise (*Illicium verum*) has been used in folk medicine of China, India, and most other Asian countries to treat stomach aches, vomiting, insomnia, dermal inflammation and rheumatic pain as well as a common spice usage. The present study was designed to investigate the possible ameliorating effect of star anise fruit extract against Etoposide-induced nephrotoxicity in rats to prove the evidence of its traditional pharmacological effectiveness. A total of 40 male Wister albino rats were divided randomly into four groups (1st group was control; 2nd group was treated with star anise, 3rd group was received Etoposide, and 4th group was treated with both star anise and Etoposide. The administration of Etoposide significantly caused elevation in creatinine, blood urea, sodium, potassium and chloride while calcium ions was significantly decrease when compared with control group. Co-treated rat with star anise and Etoposide maintained the levels of the measured some parameters (creatinine, chloride and calcium ions) closer to the normal values while urea, sodium and potassium ions were significantly decrease when compared with Etoposide group. Histopathological evidence, together with observed Ki67-ir, supported the detrimental effect of Etoposide and the ameliorating effect of star anise water extract on renal toxicity. Finally, it could be concluded that star anise has a promising role and it worth to be considered as a natural substance for ameliorating the renal toxicity and injury induced by Etoposide chemotherapy.

Keywords: Etoposide; *Illicium verum*; kidney functions; electrolytes; histology, immunohistochemistry; rat kidney.

1 Introduction

Etoposide is a semi-synthetic compound derived from the plant podophyllum pelltatum and are antineoplastic agents long been used for treatment of human malignancies (Gordaliza et al., 2004; Pratibha et al., 2012). Etoposide, a topoisomerase II inhibitor, is used for the treatment of ovarian, testicular, lung, stomach, bladder, prostate and uterine cancers (Sweetman, 2002). It is 4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-β-D glucopyranoside].

Star anise fruits are traditionally used in Chinese medicine to treat stomach aches, vomiting, insomnia, dermal inflammation and rheumatic pain as well as a common spice usage (Editorial Committee of Chinese Pharmacopoeia, 2010). The active compounds of star anise crude extracts possess wide pharmacological properties, such as antimicrobial, antioxidant, insecticidal, pain killer and sedative effects. In addition, it is the main source of shikimic acid, which is a primary ingredient of Tamiflu (Wang et al., 2011).

Etoposide does not distinguish between the cancer and normal cells and hence it eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body. Therefore; the present study was conducted to examine the possible modifying effects of star anise aqueous extract against kidney toxicity,

injury and Ki67 alterations induced by etoposide in male rats.

2. Materials and methods

The experiments were performed on 40 male rats weighing 130±10g and of 10 week's age. The rats were kept in our Faculty animal house for one week before the experimental work and maintained on a standard rodent diet and water available *ad libitum*. After one weeks of acclimation, rats were equally divided into four groups. The 1st group was control group included rats received no treatment while the 2nd group was star anise group included rats received star anise powdered suspension in distilled water (SA; 100 mg/kg BW) daily by oral gavages for four weeks. The 3rd group was the etoposide group included rats that injected interprotinally with Etoposide (1mg/kg B.W/2 day) orally for four weeks (Kamble et al., 2013). The 4th group was the group treated that with star anise plus Etoposide for four weeks.

2.1. Kidney functions and electrolytes

At the end of the experimental period, animals were fasted overnight and for clinical chemistry, blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes for estimation of urea according to the method of Patton and Crouch (1977), creatinine and sodium and potassium levels according to the method of Tietz (1983).

2.2 Histopathological evaluation

Immediately after decapitation rats were dissected, kidney from different groups were quickly removed, washed in 0.9 saline solutions and fixed in 10 % neutral buffered formalin. After fixation, specimens were dehydrated, cleared and embedded in molten paraffin. Kidney sections of 7 microns thickness were cut, mounted on clean slides and stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Bancroft and Stevens (1990).

2.3 Immunohistochemical detection of KI67:

Expression of Ki67 immunoreactivity (Ki67-ir) was detected using avidin Biotin Complex (ABC) method. The sections were incubated with anti-mouse Ki67 monoclonal antibody (dilution 1:50, DAKO Japan Co, Ltd, Tokyo, Japan) for 1-2 hours at room temperature.

2.4 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 kidney damage

Data presented in Table (1) showed that rats treated with Etoposide caused significant increase ($P < 0.05$) in serum urea, creatinine, sodium, potassium and chloride while calcium ions was significantly decrease when compared with control group. Co-treated rat with star anise and Etoposide maintained the levels of the measured some parameters (creatinine, chloride and calcium ions) closer to the normal values while urea, sodium and potassium ions were significantly decrease when compared with Etoposide group.

Kidney histopathology

Regarding the histopathological examination of the kidney from the rats control and star anise groups showed normal renal cortex and medulla with normal histological features i.e. normal structure 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. Bundles of parallel tubules can be identified running into the cortex (Figures 1A&1B).

Kidney sections in Etoposide group revealed marked glomerular injury, moderate glomeruli atrophy, moderate lymphocytic infiltration and degeneration in renal tubular epithelial cells (Figure 1C). Kidney sections of co-treated Etoposide with star anise revealed mild glomeruli atrophy and mild to moderate cellular infiltration in renal tubular cells (Figure 1D).

Ki67 immuohistochemical changes:

The detection and distribution in Ki67 immunoreactivity (Ki67-ir) in kidney sections in the different groups under study were revealed in Figures 2A-2D.

Kidney sections in control and star anise groups show moderate positive reaction for Ki67-ir (grade 3) in both glomeruli and renal tubules nuclei (Figures 2A&2B).

In contrast mild positive reactions were detected for Ki67-ir (grade 1) in the kidney sections in Etoposide rats group (Figure 2C). While, a moderate positive reaction for Ki67-ir (grade 2) were observed in

kidney sections of co-treated Etoposide with star anise (Figure 2D).

Table 1: Changes in kidney functions and electrolytes in different groups.

Parameters	**Control**	**star anise**	**Etoposide**	**star anise +Etoposide**
Urea (U/l)	32.9±1.23^{b}	29.7±1.65^{b}	45.5±2.91^{a}	41.5±1.84^{a}
Creat (U/l)	0.67±7.037^{b}	0.59±0.02^{b}	0.91±0.044^{a}	0.68±0.051^{b}
Na (U/l)	134.7 ±7.56^{b}	135±7.06^{b}	148.3±8.50^{a}	141±7.99^{ab}
K (g/dl)	4.43±0.35^{b}	4.76±0.041^{b}	6.15±0.43^{a}	5.75±0.632^{ab}
Ca (g/dl)	0.936±0.036^{b}	0.931±0.033^{b}	0.769±0.072^{a}	0.919±0.041^{b}
cl (g/dl)	100.5±4.44^{b}	100.9±5.09^{b}	114.1±8.61^{b}	103±9.55^{a}

Data are expressed as mean ± SE of 10 observations. Superscripts of different letters differ significantly ($p<0.01$) from each other. ^{b}Significantly different from Etoposide group. ^{a}Significantly different from control group.

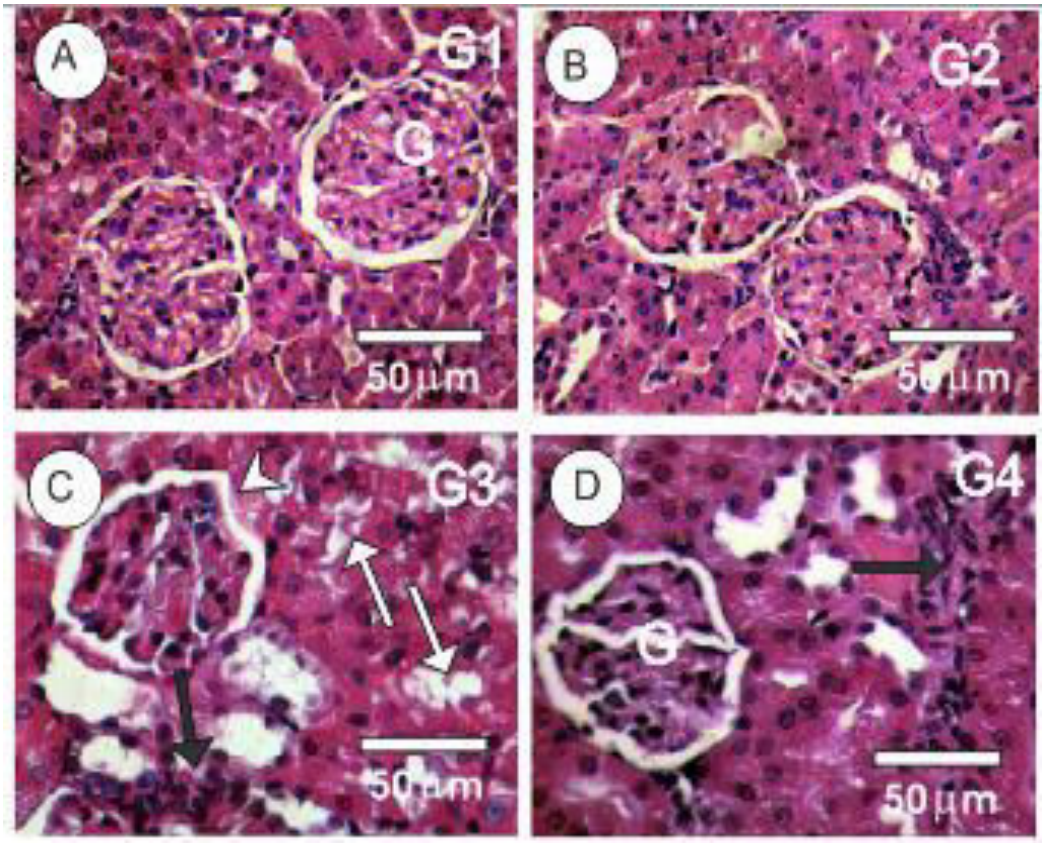
Discussion

Kidney is an important targeted organ for xenobiotic compounds that produce renal toxicity including tubular cells and glomerulus (Mohamed et al., 2003). These compounds inhibit the incorporation of amino acid into protein causing an increase in urea levels which is the major nitrogen-containing metabolic product of protein metabolism (Pollak and Harsas, 1982).

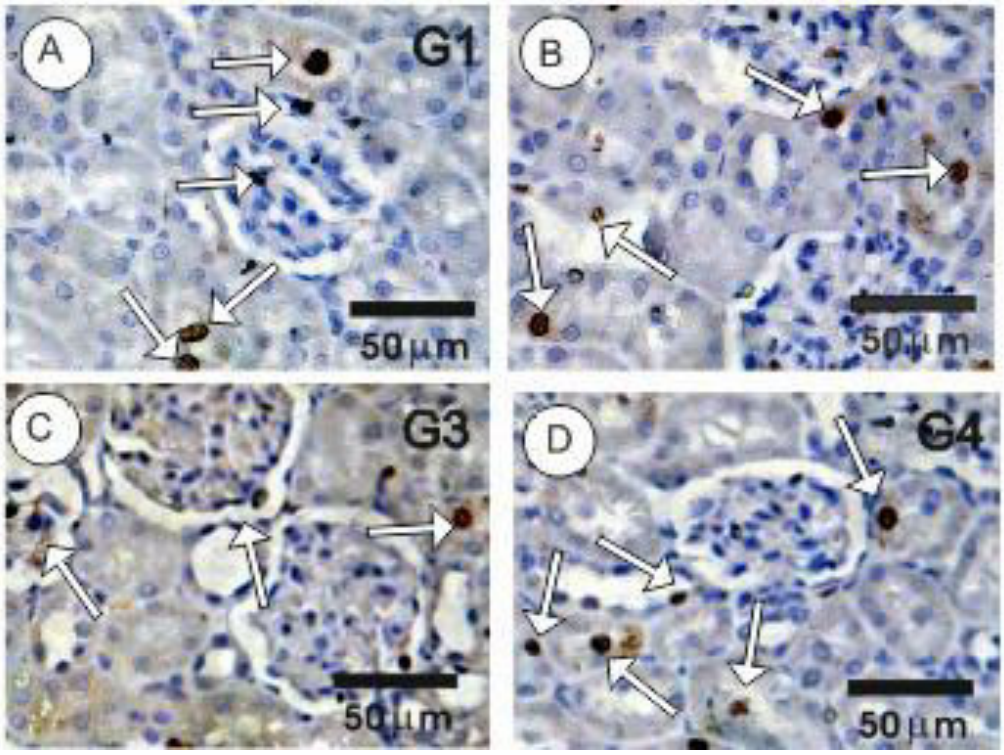
Although a number of studies have demonstrated some side-effects of the chemotherapeutic drugs, the current work aimed to study the possible modifying effects of rosemary extract against both kidney toxicity induced by Etoposide in male albino rats.Our study revealed that there are no symptoms of morbidity or mortality reported after oral administration of star anise extracts in doses up to 100 mg/kg in rats suggesting that star anise extracts were safe to be used and are nontoxic.

In the current study, a significant elevation in serum urea, creatinine, sodium, potassium and chloride while calcium ions were significantly decrease in Etoposide group. The elevation in serum urea, creatinine, sodium and potassium levels in Etoposide-treated rats is considered as a significant marker of renal dysfunction and it may be related to metabolic disturbances in liver function, as urea is the end-product of protein catabolism Administration of rosemary as co-treatments protects the kidney function from Etoposide intoxication as indicated by significant restoration of serum urea and creatinine. Moreover, elevated blood urea is known to be associated with the elevation of mammalian protein catabolism and/or the conversion of ammonia to urea as a result of elevated arginase enzyme synthesis which involved in the production of urea. Furthermore, xenobiotics intensify the acid-secretory function of kidney and change the transport of sodium (Rudenko et al., 1998). This result is in harmony with Saleh et al. (2014) and Basuony et al. (2015) who reported that, Cisplatin increased urea, creatinine and potassium. Also; Basuony et al. (2015) not agreed with our results and reported that; sodium ions were significantly decrease after Cisplatin injections. In contrast, administration of star anise as co-treatments maintained the levels of urea, creatinine, potassium and sodium to the normal values.

In the current study, a marked glomerular injury, moderate glomeruli atrophy, moderate lymphocytic infiltration and degeneration in renal tubular epithelial cells on the Kidney sections in Etoposide group. Our result is agree with Saleh et al. (2014) and Basuony et al. (2015) who reported that; Cisplatin increased urea, creatinine and uric acid activities that causes nephrotoxicity.



Figures 1: A-D: Photomicrographs of rat kidney sections in the different experimental groups stained with Haematoxylin & Eosin. **A&B:** Rat kidney sections in control and star anise groups revealed normal kidney structure with normal renal cortex and medulla with normal structure for glomeruli (G) and the Bowman's capsule. **C:** Kidney sections in Etoposide group revealed marked glomerular injury, moderate glomeruli atrophy, moderate lymphocytic infiltration (Black arrows) and degeneration in renal tubular epithelial cells (White arrows). **E:** Kidney sections of co-treated Etoposide with star anise revealed mild glomeruli atrophy and mild to moderate cellular infiltration in renal tubular cells (Black arrows).



Figures 2: A-D: Photomicrographs of rat kidney sections in the different experimental groups stained with Ki67-ir. **A&B**: Kidney sections in control and star anise groups show moderate positive reaction for Ki67-ir (arrows) in both glomeruli and renal tubules nuclei. **C:** kidney sections in Etoposide rats group showed mild positive reactions were detected for Ki67-ir. **D:** Moderate positive reaction for Ki67-ir were observed in kidney sections of co-treated Etoposide with star anise (arrows).

Our results are disagreement with Cetiner et al. (2005) who reported that chemotherapy treatment has a non-significant change in creatinine level. In agreement with the present study, Cummings and Schnellmann (2002) and Pabla and Dong (2008) reported that cisplatin chemotherapy exposure leads to proximal and distal tubular region damage in the kidney. Our results agreed with Tousson et al. (2014) who find that ginseng extract acts a protective and ameliorated effect on MTX-induced hepatic and renal toxicity.

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 Gerdes et al. (1983). 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 (Gerdes et al., 1984). The Ki-67 staining and tubule histology suggest a substantial diminution in tubule regeneration in mice and suppression of macrophages during their repair phase (Lee et al., 2011). In the current study; significant decrease in KI67 expression in kidney sections after treatment with

Etoposide when compared with control. In contrast, co-treatment Etoposide with star anise increases the depletion of Ki67-ir in kidney. Our histopathological and immunohistochemical results showed that, treatment of rat kidney with aqueous extract of star anise showed moderate to good degree of improvement in the malpighian corpuscles and renal tubules in kidney sections in Etoposide group.

The significant restoration of all of the above biochemical and histopathological parameters towards normal values upon star anise extract treatment tested in the present study indicates the protection of vital organs such as kidney from damage induced by Etoposide. Hence, the present study confirms the potent renal protective and antioxidant nature of active phenolic compounds in star anise 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 star anise extracts.

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