GSC Biological and Pharmaceutical Sciences, 2019, 07(03), 008–020

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Contribution of garlic for improving the cytoprotective effect of mesna against cyclophosphamide toxicity in rats

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Publication history**:** Received on 16 May 2019; revised on 27 May 2019; accepted on 03 June 2019

Article DOI: https://doi.org/10.30574/gscbps.2019.7.3.0089

Abstract

Cyclophosphamide (CYP), an oxazophosphorine- alkylating agent, is a widely used drug for treatment of neoplastic and severe autoimmune diseases. Adverse effects of CYP restrict its therapeutic benefits. Mesna (MS), a thiol compound, is used as a protective agent against hemorrhagic cystitis induced by CYP. This study aimed to investigate the potential role of raw garlic homogenate (RGH) for improving the protective effect of mesna against CYP toxicity in rats. Thirty male albino rats were divided into five equal groups. Control group, CYP group (a single i.p. dose, 200 mg/kg b.wt.), CYP+MS group (a total dose of 120 mg/kg b.wt. in three equal doses, i.p.), CYP+RGH group (500 mg/kg b.wt., orally, once daily 5 days before and after CYP injection) and CYP+MS+RGH group. CYP induced hematological changes, including significant reduction of RBCs, Hb, PCV with thrombocytopenia and leukocytopenia. Also, significant increase in serum MDA content concomitantly with significant reduction in TAC was recorded. This was associated with histopathological alterations in the examined tissues. However, mesna and/or garlic improved the recorded hematological, biochemical and histopathological alterations induced by CYP with marked improvement using their combination. In conclusion, garlic supplementation in combination with mesna could be of a great value to introduce therapeutic strategies for patients undertaking cyclophosphamide therapy.

Keywords: Cyclophosphamide; Garlic; Mesna; Hematology; Histopathology

1. Introduction

Cyclophosphamide (CYP) is an alkylating agent and widely used anti-neoplastic drug for the treatment of malignant lymphomas, myeloma, leukemia, neuroblastoma, adenocarcinoma, retinoblastoma and breast carcinoma [1] and as a preparatory treatment for bone marrow transplantation [2]. Because of its immunosuppressive activity, it is also frequently used as an immunosuppressive agent for organ transplantation [3] and for treating autoimmune diseases [4]. Also, it used for treatment of amyloidosis, idiopathic nephritis, severe rheumatoid arthritis and multiple sclerosis [5,6]. Most of these treatment regimens require high doses of CYP that are associated with adverse effects such as hemorrhagic cystitis; hematological, immunological, cardiac, hepatic and renal toxicities [7-11].

Mesna (sodium-2-mercaptoethane-sulfonate) is a thiol compound that used clinically as a protective agent against the toxicity of chemotherapy and as a regional detoxificant to reduce the incidence of haemorrhagic cystitis and haematuria in patients received CYP [12]. Furthermore, mesna exerts anti-inflammatory, anti-oxidative and anti-apoptotic properties [13]. Mesna is not able to perfectly counteract the side effect of CYP, and studies since its discovery have investigated the use of alternatives to increase its efficacy. Natural herbs have been used to treat various diseases and

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provided as chemotherapeutic agents to protect against toxic side effects mainly due to their antioxidant activities [14, 15].

Garlic (*Allium sativum*) is a worldwide traditional food and dietary supplement. Nowadays, several garlic preparations including fresh or aged garlic extracts, garlic powder and garlic oil are used as a remedy for a variety of diseases. Raw garlic homogenate is the most frequently used garlic preparation for scientific studies, because it is the most consumed garlic [16].

Garlic contains at least 33 sulfur compounds, amino acids, minerals and vitamins [17]. The chemical constituents of garlic have been recommended for treatment of cardiovascular disease, cancer, diabetes, blood pressure, atherosclerosis and hyperlipidaemia. Moreover, the sulfur compounds of garlic have antioxidant actions by scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells [18]. Cyclophosphamide is able to generate ROS and induce oxidative damage. Thus, strategies to minimize the side effects of chemotherapeutic agents while preserving their efficacy are needed. In the light of this, the current study aimed to investigate the potential role of raw garlic homogenate for improving the protective effect of mesna against cyclophosphamide toxicity in rats.

2. Material and methods

2.1. Experimental Animals

A total of thirty healthy male albino rats (195-200 g) were purchased from Al-Zyade Experimental Animals Production Center, Giza, Egypt. Animals were housed in polypropylene cages with mesh wire tops and kept in a natural ventilated room 26±3 °C, 45-60% relative humidity, natural daily dark/light cycle, provided with standard commercial diet and clean tap water *ad libitum*. Rats were acclimatized to the laboratory environmental conditions for 2 weeks before the beginning of the experiment.

2.2. Chemicals

Cyclophosphamide (Endoxan® 200 mg, Baxter Oncology GmbH, Germany), was dissolved in 5ml sterile distilled water to a concentration of 40 mg/ml immediately before treatment and mesna (Uromes® 400 mg/ 4ml, EMIC United Company) were purchased from local pharmacy. Diagnostic kits for assaying serum biochemical parameters were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. Other utilized chemicals were of analytical grade and commercially available.

2.3. Preparation of raw garlic homogenate

Fresh garlic was collected from local market. The cloves were cleaned, peeled, sliced, ground into a paste and a homogenate was made in distilled water. Freshly prepared extract at concentration of 0.2 mg/ml (corresponding to 500 mg/kg b.wt.) was orally administrated to rats within 30 min after preparation [19].

2.4. Experimental design and animal grouping

Rats were weighed and randomly allocated into five equal groups. Control group, rats received only the standard diet and tap water. CYP group, rats received a single i.p. dose, 200 mg/kg b.wt. at 6th day of the experiment [20]. CYP+MS group, rats received CYP and mesna as a total dose of 120 mg/kg b.wt., divided into three i.p. equal doses, 20 min before and 4, 8 hours after CYP injection [21]. CYP+RGH group, rats received CYP and RGH oral administration (500 mg/kg b.wt.), once daily 5 days before and after CYP injection [19]. CYP+MS+RGH, rats received CYP, MS and RGH as previously mentioned (Table 1).

Table 1: The experimental design and animal grouping.

Treatment schedule / Group	Days of experiment							Day of sacrifice (the 12th day)
	Before CYP injection	Day of CYP injection at the 6th day of the experiment					After CYP injection	
	5 days (1st - 5th day)	-60 min	-20 min	0	+4 h	+8h	5 days (7th - 11th day)	
Control	----------	------	-------	------	----	-----	----------	
CYP	----------	-----	-----	CYP	----	-----	----------	
CYP + MS	----------	-------	MS	CYP	MS	MS	----------	
CYP + RGH	RGH	RGH	------	CYP	----	-----	RGH	
CYP + MS + RGH	RGH	RGH	MS	CYP	MS	MS	RGH	

CYP: Cyclophosphamide; MS: Mesna; RGH: Raw garlic homogenate

2.5. Collection of samples

At the 12th of the experiment, rats were fasted overnight, anaesthetized and blood samples were collected from the medial canthus of the eye. Blood samples were divided into two parts. The first part was collected on EDTA for hematological analysis, while the other part was used for serum collection and was stored at -20 ºC until be used for biochemical analysis. Immediately after blood collection, rats were rapidly sacrificed and tissue specimens from the urinary bladder, liver, kidney, heart and spleen were collected and fixed in 10% neutral buffered formalin solution for histopathological examination.

2.6. Hematological examination

Estimation of red blood cells (RBCs) count, hemoglobin (Hb) concentration, packed cell volume (PCV) %, total (TLC) and differential leukocytic counts were measured according to the routine hematological procedures described by Weiss and Wardrop [22]. Mean corpuscular volume (MCV) was calculated as PCV divided by red cell count and multiplied by 10. Mean corpuscular hemoglobin (MCH) was calculated as hemoglobin divided by red cell count and multiplied by 10, while mean corpuscular hemoglobin concentration (MCHC) was calculated as hemoglobin divided by PCV multiplied by 100.

2.7. Serum oxidant/ antioxidant biomarkers

Serum Malondialdehyde (MDA) content and total antioxidant capacity (TAC) were estimated according to Ohkawa et al. [23] and Koracevic et al. [24], respectively, using commercial kits.

2.8. Histopathological examination

The formalin fixed specimens were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate and embedded in paraffin after having completed the routine follow-up steps. Sections at 3-5 µ sections were obtained from urinary bladder, liver, kidney, heart and spleen using microtome (LEICA RM 2135) and stained by hematoxylin and eosin (H&E) stain for light microscopical investigation according to Bancroft and Gamble [25]. Photos were taken using digital camera (LEICA DMLB Germany).

2.9. Statistical analysis

Values are presented as mean ± standard error. Statistical analysis of data was carried out using analysis of variance by one-way ANOVA test followed by Duncan multiple comparison tests. All data were statistically analyzed using statistical software program SPSS (Statistical package for Social Sciences) Version 16 released on 2007.

3. Results

3.1. Effects on animals' general condition

Control rats did not exhibit any clinical manifestations. However, dullness, depression, lethargy, rough hair coat, dehydration were the observed signs of CYP- treated group. Other groups exhibited less sever clinical manifestations.

3.2. Effect on hematological parameters

Compared to control group, rats of the CYP- treated group revealed significant ($P<0.05$) decreases of RBCs count, Hb, PCV without any significant changes in MCV, MCH and MCHC levels along with thrombocytopenia. Moreover, CYP induced significant reduction in WBCs, neutrophils and lymphocytes with insignificant reduction in eosiniphils, basophils and monocytes. However, administration of mesna and/or garlic improved these values with marked improvement in their combination (Table 2).

Table 2 Hematological parameters in control and different treated groups

Parameters	Control	CYP	CYP + MS	CYP + RGH	CYP+MS+RGH
RBCs ($x10^6$/µl)	8.39 ± 0.31^{a}	4.70 ± 0.19^{d}	6.73 ± 0.27^{b}	5.57 ± 0.22^{c}	7.70 ± 0.25^{a}
PCV (%)	44.87 ± 3.56^{a}	27.59 ± 1.70^{c}	39.05 ± 1.65^{ab}	33.99 ± 1.08^{bc}	42.82 ± 3.11^{a}
Hb (g/dl)	14.30 ± 0.61^{a}	9.67 ± 0.44^{d}	12.10 ± 0.41^{bc}	10.80 ± 0.25^{cd}	12.83 ± 0.57^{ab}
MCV (fl)	53.29 ± 2.39^{b}	55.49 ± 2.26^{ab}	61.07 ± 1.68^{a}	58.05 ± 0.49^{ab}	58.55 ± 1.33^{ab}
MCH (pg)	16.71 ± 0.91^{b}	19.41 ± 0.52^{ab}	18.03 ± 0.78^{ab}	20.68 ± 1.68^{a}	17.04 ± 0.31^{b}
MCHC (%)	30.31±2.79	31.79±0.28	31.05±1.18	35.48±3.76	32.11±1.69
Platelets ($x10^3$/ µl)	491 ± 9.70^{a}	196 ± 9.40^{d}	332 ± 9.23^{c}	397 ± 8.84^{b}	472 ± 8.04^{a}
TLC ($x10^3$/ µl)	9.06 ± 0.19^{a}	3.46 ± 0.32^{d}	5.50 ± 0.58^{c}	6.86 ± 0.45^{b}	8.64 ± 0.27^{a}
Neutrophils ($x10^3$/ µl)	3.20 ± 0.095^{a}	1.55 ± 0.13^{c}	1.79 ± 0.16^{c}	2.40 ± 0.23^{b}	2.86 ± 0.18^{ab}
Lymphocytes ($x10^3$/µl)	5.30 ± 0.04^{a}	1.59 ± 0.15^{c}	2.91 ± 0.48^{b}	3.72 ± 0.35^{b}	4.65 ± 0.23^{a}
Eosinophils ($x10^3$/ µl)	0.18 ± 0.01^{bc}	0.03 ± 0.02^{c}	0.73 ± 0.04^{a}	0.35 ± 0.21^{abc}	0.62 ± 0.23^{ab}
Basophils ($x10^3$/ µl)	0.02 ± 0.003^{b}	0.21 ± 0.003^{b}	0.01 ± 0.006^{b}	0.01 ± 0.006^{b}	0.05 ± 0.01^{a}
Monocytes ($x10^3$/ µl)	0.35 ± 0.06^{ab}	0.28 ± 0.09^{ab}	0.20 ± 0.05^{b}	0.38 ± 0.05^{ab}	0.46 ± 0.02^{a}

Values are presented as mean ± SE (n= 5). Means with different superscripts in the same raw are significantly different at ($P<0.05$).
CYP: Cyclophosphamide; MS: Mesna; RGH: Raw garlic homogenate. RBCs, red blood cell count; Hb, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; TLC, total leukocyte count.

3.3. Oxidant/ antioxidant biomarkers

Administration of CYP to rats significantly increased ($P<0.05$) serum MDA content concomitantly with reduction in TAC in compared to control group. Rats administered mesna or garlic significantly abolished these changes and restored the normal control values in rats administered a combination of MS and RGH (Table 3).

Table 3 Serum oxidant/ antioxidant biomarkers in control and different treated groups

Parameters	Control	CYP	CYP + MS	CYP + RGH	CYP+ MS+ RGH
MDA (nmol/ml)	4.23 ± 0.17^{b}	8.47 ± 0.29^{a}	6.57 ± 0.39^{c}	6.34 ± 0.27^{c}	4.92 ± 0.11^{b}
TAC (mmol/ ml)	1.48 ± 0.17^{a}	0.62 ± 0.06^{b}	0.92 ± 0.05^{c}	0.95 ± 0.02^{c}	1.41 ± 0.12^{a}

Values are presented as mean ± SE (n= 5). Means with different superscripts in the same raw are significantly different at ($P<0.05$).
CYP: Cyclophosphamide; MS: Mesna; RGH: Raw garlic homogenate. MDA, Malondialdehyde; TAC, total antioxidant capacity.

3.4. Histopathological findings

3.4.1. Microscopic alterations of urinary bladder

The transitional epithelium and basement membrane that separates the epithelium from the underlying lamina propria of the bladders were preserved in control group (Fig.1 A). In CYP- treated rats, there were epithelial desquamation, reactive hyperplasia of urinary epithelium, dilatation and congestion of bladder vessels with leukocyte infiltration, hemorrhage and edema in the connective tissues that constituted the lamina propria (Fig.1 B1,2). However, rats administered CYP and mesna showed only mild connective tissue edema and the uroepithelium thickness was diminished and no uroepithelial erosion or ulceration was observed (Fig.1 C). Similarly, CYP+RGH- treated rats showed that uroepithelium thickness was preserved with mild uroepithelial desquamation and mild congestion of blood vessels without connective tissue edema (Fig.1 D). Nearly normal urinary bladder histology was observed in CYP+MS+RGH-treated rats (Fig.1 E).

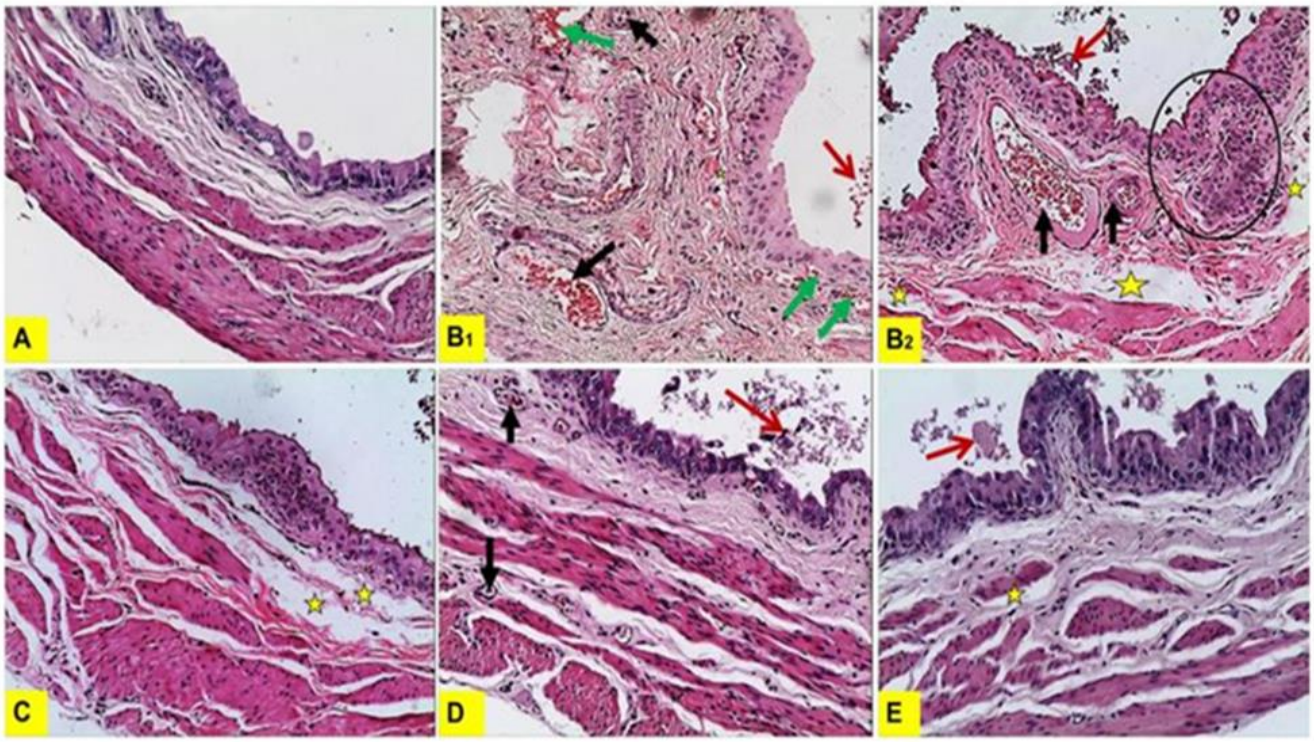

Figure 1 Photomicrographs of urinary bladder sections of rats in different groups (H&E stain X_{10}) (star: edema, red arrow: uroepithelial desquamation, black arrow: congestion, green arrow: hemorrhage, circle: reactive hyperplasia of urinary epithelium) A: Control group showing normal histology. B1, 2: CYP group showing uroepithelial desquamation, reactive hyperplasia of urinary epithelium, dilatation and congestion of bladder vessels with leukocyte infiltration and edema. C: CYP+MS group showing uroepithelial preservation and mild edema. D: CYP+RGH group showing uroepithelial preservation and mild congestion of blood vessels. E: CYP+MS+RGH group showing somewhat normal histology and uroepithelial preservation with mild edema.

3.4.2. Microscopic alterations of liver

Control group showing normal hepatic architecture (Fig. 2 A). However, CYP- treated rats showed diffuse granular degeneration of most hepatocytes, edema, congestion of central vein and hepatic sinusoids, inflammatory cells infiltration, hepatic cells with nuclear pyknosis, some apoptotic hepatic cells and multifocal areas of coagulative necrosis infiltrated by inflammatory cells. (Fig.2 B1, 2). Liver sections of CYP+MS- treated rats showed congestion of hepatic sinusoids, some hepatic cells with nuclear pyknosis (Fig.2 C). While, CYP+RGH- treated rats showed mild congestion of central vein and hepatic sinusoids and small area of coagulative necrosis infiltrated by some inflammatory cells. (Fig.2 D). No histological change was observed in liver of CYP+MS+RGH- treated rats (Fig. 2 E).

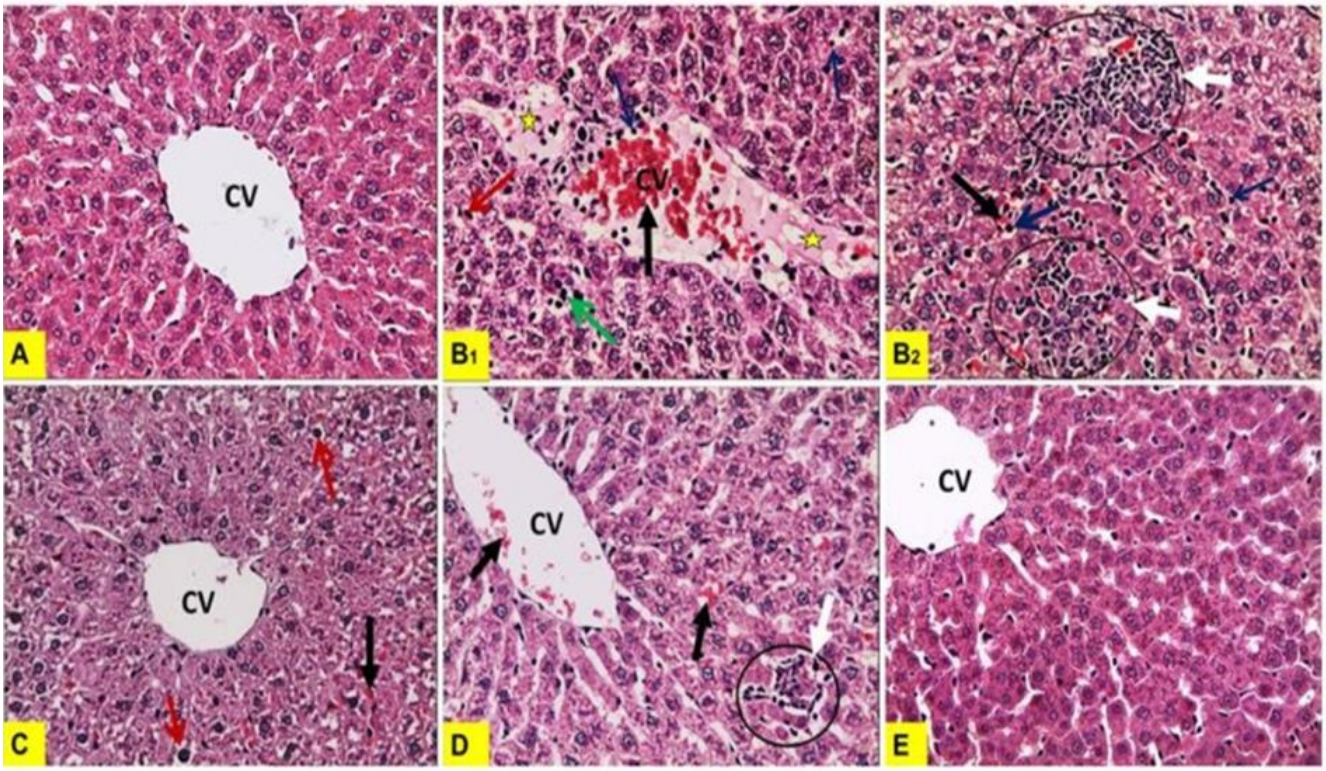

Figure 2 Photomicrographs of liver sections of rats in different groups (H&E stain X20) (star: edema, black arrow: congestion, red arrow: nuclear pyknosis, blue arrow: inflammatory cells infiltration, green arrow: apoptotic hepatic cells, white arrow and circle: area of coagulative necrosis infiltrated by inflammatory cells) A: Control group showing normal tissue architecture of liver. B1,2: CYP group showing edema, congestion of central vein and hepatic sinusoids, inflammatory cells infiltration, hepatic cells with nuclear pyknosis, some apoptotic hepatic cells and multifocal areas of coagulative necrosis infiltrated by inflammatory cells. C: CYP+MS group showing congestion of hepatic sinusoids, some hepatic cells with nuclear pyknosis. D: CYP+RGH group showing mild congestion of central vein and hepatic sinusoids, small area of coagulative necrosis infiltrated by some inflammatory cells. E: CYP+MS+RGH group showing: somewhat normal histological structure of liver parenchyma**.**

3.4.3. Microscopic alterations of kidney

Normal renal tissue was observed in control group (Fig.3 A). CYP- treated rats showed interstitial edema, marked glomerular congestion, sever glomerular and interstitial hemorrhage, glomerular atrophy with nuclear pyknosis of glomerular cells, dilated renal tubules with cloudy swelling and intraluminal desquamated epithelial cells (Fig.3 B1,2). Kidney of CYP+MS- treated rats showed mild glomerular congestion, glomerular atrophy with nuclear pyknosis of glomerular cells, dilatation of some renal tubules with cloudy swelling (Fig.3 C). While, CYP+RGH- treated rats showed mild congestion of glomeruli, slight interstitial hemorrhage and most renal tubules were apparently normal (Fig.3 D). Somewhat normal intact tubules and glomerular tufts with no observable histological changes were observed in CYP+MS+RGH- treated rats (Fig.3 E).

3.4.4. Microscopic alterations of heart

Rats of control group showing normal myocardial muscles and interstitial tissue (Fig.4 A). Hearts of CYP- administrated rat showed loss of striation of myocardial muscles, hemorrhage, edema and lymphocytic infiltration between myocardial muscles in addition to myocardial necrosis and cardiomyolysis with granular appearance of sarcoplasm and myofibers (Fig.4 B1,2). Heart of CYP+MS (Fig.4 C) and CYP+RGH (Fig.4 D) groups showed mild hemorrhage, edema between myocardial muscles beside necrosis in some myocardial muscles were observed. CYP+MS+RGH- treated rats showed somewhat normal histology except single cell necrosis myocardial muscle (Fig.4 E).

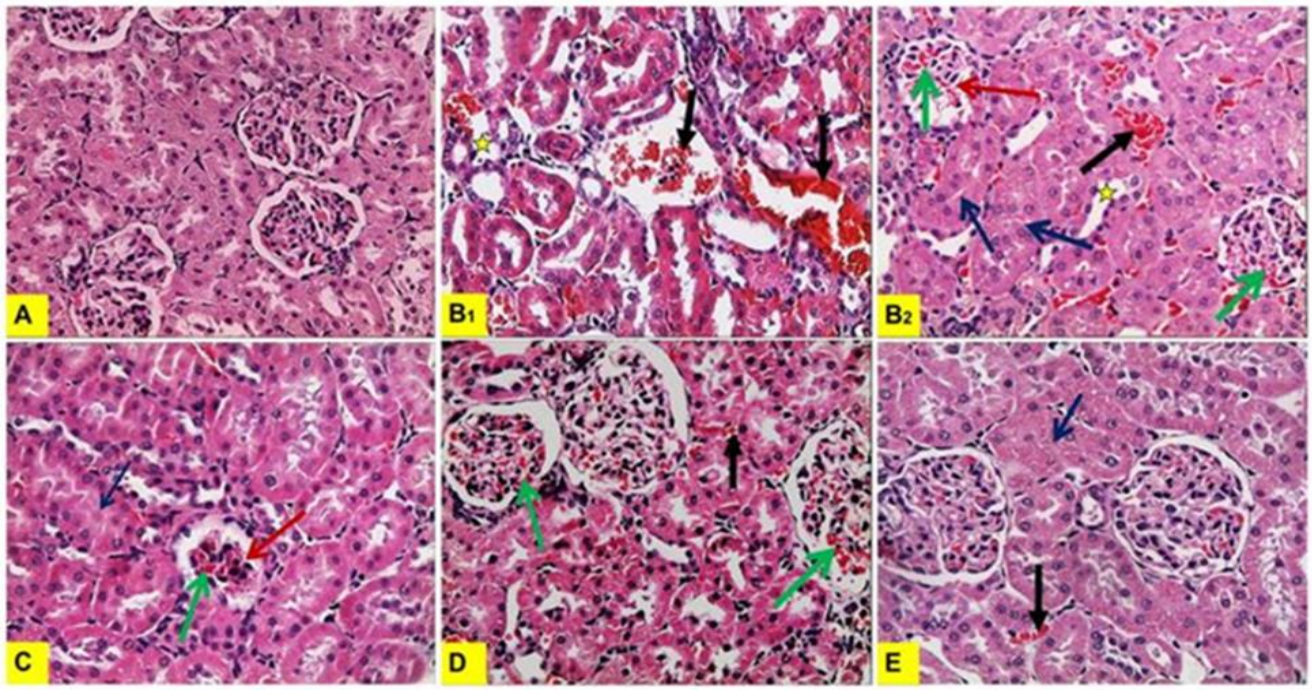

Figure 3 Photomicrographs of kidney sections of rats in different groups (H&E stain X20) (star: edema, black arrow: hemorrhage, red arrow: glomerular atrophy with nuclear pyknosis of glomerular cells, blue arrow: dilated renal tubules with cloudy swelling) A: Control group showing normal tissue architecture of kidney. B1,2: CYP group showing glomerular congestion, glomerular and interstitial hemorrhage, glomerular atrophy with nuclear pyknosis of glomerular cells, dilated renal tubules with cloudy swelling, interstitial edema. C: CYP+MS group showing glomerular congestion, glomerular atrophy with nuclear pyknosis of glomerular cells, dilated renal tubules with cloudy swelling. D: CYP + RGH group showing congestion of glomeruli, slight interstitial hemorrhage. E: CYP+MS+RGH group showing somewhat normal histological structure of renal tissue.

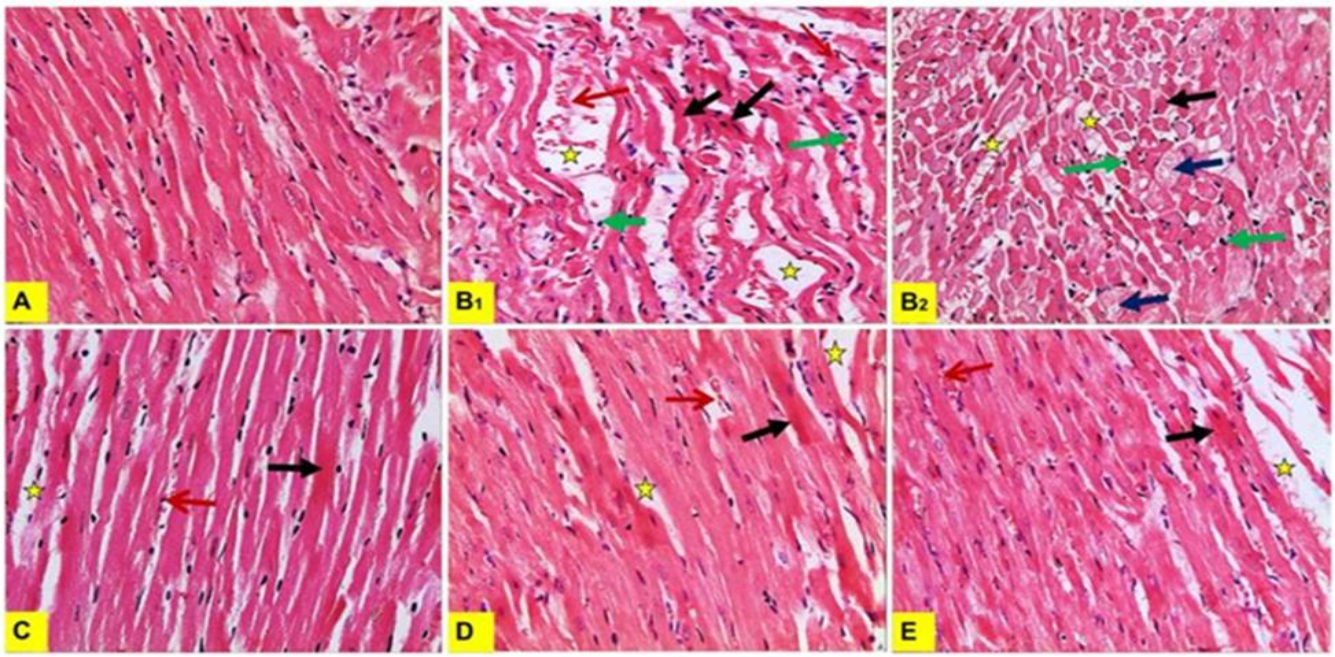

Figure 4 Photomicrographs of heart sections of rats in different groups (H&E stain X20) (star: edema; red arrow: hemorrhage, green arrow: lymphocytic infiltration, black arrow: Zenker's necrosis, blue arrow: cardiomyolysis) A: Control group showing: Normal histology. B1,2: CYP group: B1 longitudinal section B2 cross section showing: loss of striation, hemorrhage, edema and lymphocytic infiltration between myocardial muscles and myocardial necrosis and cardiomyolysis with granular appearance of sarcoplasm and myofibers. C: CYP+MS group, D: CYP+RGH group showing

mild hemorrhage, edema between myocardial muscles beside necrosis in some myocardial muscles. E: CYP+MS+RGH group showing: somewhat normal except single cell necrosis myocardial muscle.

3.4.5. Microscopic alterations of spleen (Fig. 5):

Histopathological examination in the spleen of control rats showed normal architecture. The splenic follicles (lymphatic nodules) in white pulp were big, intact with eccentric follicular arterioles and contain small deeply basophilic lymphocyte aggregation (Fig. 5 A). Several histopathological changes were recorded in spleen of CYP- treated group, including progressive loss of white pulp and relative increases in red pulp also marked loss in distinction between white and red pulps was noted, the lymphatic nodules were dispersed with decrease in its lymphocyte population and marked loss in the chromatin of their nuclei and most of the lymphocytes contained pyknotic nuclei, the reticular cells in the red pulp were increased in number and size and their nuclei were fragmented or necrotic and increase in the size of megakaryocytes with many nuclei were observed in CYP- treated rats (Fig. 5 B1,2). Little histopathological alterations and marked improvement in the splenic tissue were noticed in CYP+MS- treated rats (Fig. 5 C). In CYP+RGH- treated rats (Fig. 5 D) and CYP+MS+RGH treated rats (Fig. 5 E) there was an obvious distinction between the white and the red pulp, the white pulp had normal accumulation of cellularity and the red pulp showed an increase in the number of the reticular cells and macrophages and most sections of spleen showed an apparently normal structure for white and red pulps as it was observed in the normal control sections.

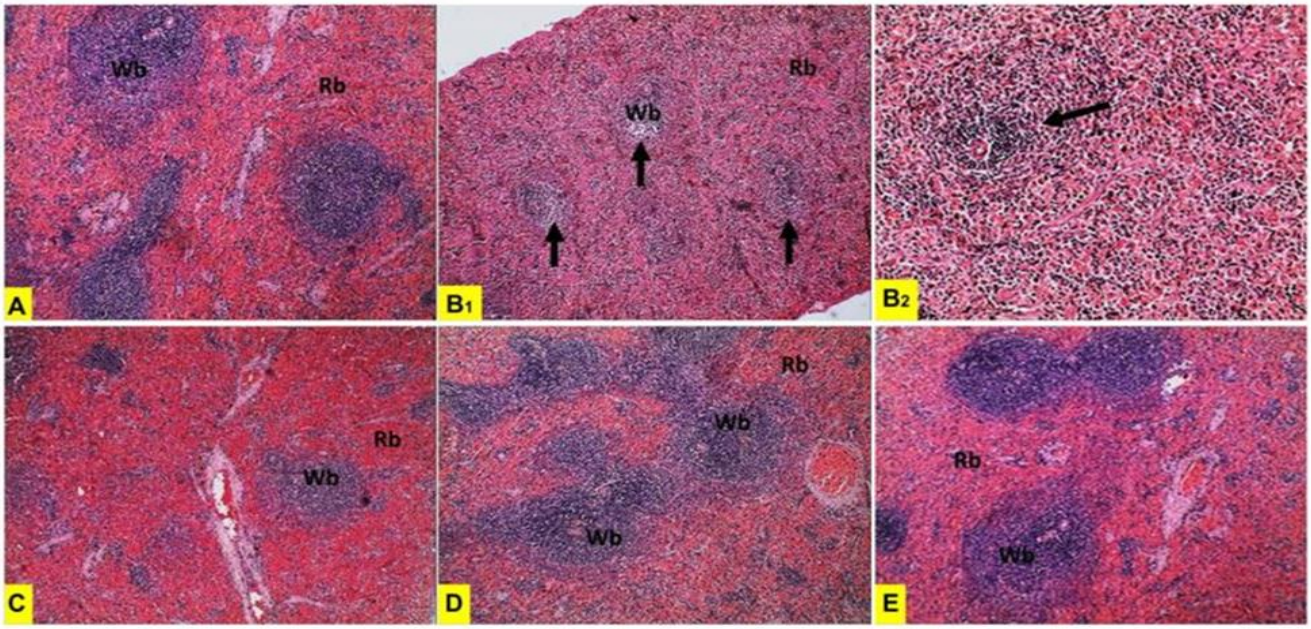

Figure 5 Photomicrographs of spleen sections of rats in different groups (H&E stain X4 ; B2 X10) (Wb: white bulb, Rb: red bulb, FA: follicular arteriole, arrow: depletion in white bulb) A: Control group showing: Normal spleen architecture. B: CYP group showing dispersed lymphatic nodules with decreased cellularity. C: CYP+MS group, D: CYP+RGH group, E: CYP+MS+RGH group showing somewhat normal structure for white and red pulps.

4. Discussion

Cyclophosphamide is an alkylating agent that is commonly used as an antineoplastic and immunosuppressive drug. Notwithstanding its chemotherapeutic properties, CYP causes oxidative systemic damages [26]. Mesna has been widely used as an effective drug against CYP adverse effects. Recently, several studies applied to study the potential ameliorative role of natural remedies to minimize chemotherapeutics toxicity without affecting their antineoplastic activities. Therefore, this study aimed to investigate the potential role of raw garlic homogenate for improving the protective effect of mesna against cyclophosphamide toxicity in rats.

Our findings revealed that single i.p. administration of CYP at a dose of 200 mg/kg b.wt. significantly decreased the levels of RBCs, Hb, PCV without any significant changes in MCV, MCH and MCHC along with thrombocytopenia. Moreover, CYP- intoxicated rats showed significant reduction in TLC, neutrophils and lymphocytes with insignificant reduction in eosinophils, basophils and monocytes.

The above hematological findings were in agreement with Cengiz [27] and Elshater et al. [28] who stated that administration of 200 mg/ kg of CYP to rat resulted in reductions in the number of erythrocyte, hemoglobin, leukocytes, thrombocytes and hematocrit. Also, administration of 50, 100, or 150 mg/kg of CYP, i.p. caused, in a dose-dependent manner, reductions in the number of RBCs, WBCs and platelets. It is well known that CYP adversely affects the hematopoietic and immune systems leads subsequently to further hematopoietic and immune dysfunction, which represented by anemia, thrombocytopenia and leukopenia [29].

Regarding the effect of CYP on oxidant/ antioxidant status, our findings showed that CYP induced oxidative damage as evidenced by significant increase in serum MDA content concomitantly with reduction in TAC after single i.p. injection of CYP. Inconsistent with our findings, Gunes et al. [30] recorded that rats treated with 100 mg/kg CYP induced significant increase in serum MDA with reduction in TAC. Moreover, previous investigations recorded the tissue oxidative damages of CYP as demonstrated by increase of MDA and reduction of antioxidants [28, 31-35].

Oxidative stress is the imbalance of oxidant/antioxidant state leading to oxidation of lipids, DNA and proteins in the cells and hence, tissue damage. Lipid peroxidation is an oxidative destruction of poly unsaturated fatty acid. MDA is one of end products of lipid peroxidation and is used to access lipid peroxidation and free radical generation along with the estimation of TAC. Oxidative stress and generation of reactive oxygen species (ROS) have been implicated in the pathophysiology of CYP toxicity [36]. CYP induces oxidative damage through decreasing the activities of the antioxidant enzymes and increases the extent of the lipid peroxidation [37].

The mechanism by which CYP induces tissue damages could be attributed it to its cytotoxic metabolites; phosphoramide mustard and acrolein. Phosphoramide mustard exerts antineoplastic effect while acrolein, is a highly reactive metabolite that causes oxidative stress implicated in the urotoxic, hepatotoxic, nephrotoxic, cardiotoxic and immunotoxic effects of CYP [28,30-32,34,38]. Also, CYP cytotoxicity may be due to the production of TNF-α, a pleiotropic cytokine inducing cell death via necrosis and apoptosis pathway [39].

The recorded oxidative biochemical changes were supported by the observed histopathological alterations of the examined tissues. The same histopathological findings were previously observed in urinary bladder [32], liver [28], kidney [35], heart [34] and spleen [31] of CYP- treated rats.

The obtained results approved the cytoprotective effect of mesna and/ or garlic against CYP toxicity that evidenced by improvement of the altered hematological, biochemical and histopathological findings with marked improvement in rats administrated a combination of mesa and garlic. In agreement with our findings, previous studies reported the protective effect of mesna [40] and garlic [41, 42] against CYP toxicity.

Mesna has been routinely used with CYP to alleviate its side effects mainly the hemorrhagic cystitis [43]. Acrolein binding and ROS scavenging properties of MS may contribute to its beneficial protective and therapeutic properties [44, 45]. Şener et al. [45] suggested that mesna, as an antioxidant and thiol-containing drug, exhibited hepatorenal protective effect against acetaminophen-induced oxidative damage. Moreover, Şener et al. [46] stated that mesna with its antioxidant and antifibrotic properties may be of potential therapeutic value for protecting the liver against biliary obstruction-induced oxidative damage. Consequently, the recorded cytoprotective effect of mesna could be attributed to its antioxidant property.

Garlic, a natural dietary substance, has been well recognized for its medicinal properties [47]. Garlic and its compounds exert antimicrobial, anti-inflammatory, anti-artherosclerotic, antihypertensive and antihyperlipidemic, antidiabetic, hepato-renoprotective, antioxidant, anti- carcinogenic and immune modulation, and various other biological properties [18]. Previous studies have reported the protective effect of garlic against doxorubicin [48], cadmium [49], acrylamide [50] and cisplatin [51] toxicities. The beneficial therapeutic value of garlic against different toxic agents was mainly via its powerful antioxidant and free radical scavenging properties [18, 50]. RGH contains numerous antioxidant organosulfur compounds, mainly S-Allylcysteine and allicin. These compounds play an important role as antioxidants [52] and exert their antioxidant actions by inhibiting lipid peroxidation, scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells [18]. Our findings approved the immune-stimulant and the antioxidant properties of garlic.

5. Conclusion

From these observations, it can be concluded that administration of mesna or garlic alone may be insufficient in the management or treatment of the various adverse effects induced by cyclophosphamide in rats. On the other hand, administration of garlic in combination with mesna completely alleviated cyclophosphamide adverse effects. Therefore, garlic supplementation in combination with mesna could be of a great value to introduce therapeutic strategies for patients undertaking cyclophosphamide therapy.

Compliance with ethical standards

Acknowledgments

All authors would gratefully thank the team of Central Lab, Faculty of Veterinary Medicine, University of Sadat City for their technical support in the implementation of this study.

Disclosure of conflict of interest

The author declares no conflicts of interest. The author had no financial grants or external fund.

Statement of ethical approval

This study was ethically approved by the International Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City.

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How to cite this article

Tahoun EA, El- Borai NB, Khalifa HK and El- Gendy H. (2019). Contribution of garlic for improving the cytoprotective effect of mesna against cyclophosphamide toxicity in rats. GSC Biological and Pharmaceutical Sciences, 7(3), 08-20.