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Rosmarinus tomentosus extracts: Antioxidant and protective effects against cyclophosphamide and buthionine sulfoximine induced bladder urotoxicity in rats

Bechr Hamrita*[1], Kamel Rouissi[2], Yosra Messai[2], Bassem Jaouadi[3], Mohamed Cherif[4], Amel Benammar Elgaaied[2]

[1]Laboratory of Molecular immuno-oncology, Faculty of medecin, Monastir, (TUNISIA)

[2]Laboratory of Genetic, Immunology and Human Pathology, Faculty of Sciences of Tunis, University of El Manar I. 2092, Tunis, (TUNISIA)

[3]Laboratory of Prokaryotic Enzymes and Metabolites, Center of Biotechnology of Sfax, (TUNISIA)

[4]Department of Urology, Charles Nicole Hospital, Tunis, (TUNISIA)

E-mail : bechrhamrita@yahoo.fr

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ABSTRACT

Cyclophosphamide (CP) is an alkylating agent and has been primarily used as an anti-neoplastic agent for the treatment of various forms of cancer witch causes toxicity by its reactive metabolites such as acrolein and phosphoramide mustard. In this study we investigated the effect of *Rosmarinus tomentosus* (Romarin) extract on urotoxicity induced by acute dose of CP and buthionine-SR-sulfoximine (BSO) using Swiss albino mice model. Modulation of toxicity was evaluated by measuring lipid peroxidation (LPO) and anti-oxidants in urinary bladder in mice. *Rosmarinus tomentosus* showed protective effect not only on LPO but also on the enzymatic anti-oxidants. CP-treated animals exhibited a significant decrease in the activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP) and catalase (CAT) when compared to the controls. Level of reduced glutathione (GSH) was also reduced with an increase in LPO in CP-treated animals. BSO treatment depicted an additive toxic effect in CP-treated animals. Pre-treatment of *Rosmarinus tomentosus* extract restored activities of all the enzymes and thus showed an overall protective effect on additive effect of CP and BSO. Restoration of GSH by extract treatment may play an important role in reversing CP-induced apoptosis and free radical mediated LPO in urinary bladder.

KEY-WORDS

Cyclophosphamide;
Buthionine-SR-sulfoximine;
Urinary bladder anti-oxidants;
Rosmarinus tomentosus.

INTRODUCTION

Cyclophosphamide (CP) is one of the most popular alkylating anticancer drugs in spite of its toxic side effects including immunotoxicity, hematotoxicity, mutagenicity it is used in anti-neoplastic therapy[1, 2]. CP is an established drug administered for the treatment of both malignant and nonmalignant conditions[2]. How-

ever, CP intake is associated with cardiac damage, hemorrhagic cystitis, vomiting, and carcinogenicity[1]. Extensive tissue damage and toxicity in the lungs as a result of CP treatment has also been documented[3]. It is also reported to cause urological cytotoxicity in bladder[3]. Previous reports have suggested acrolein (products of CP hydroxylation in the liver) as the causal agent for bladder epithelial damage[4, 5]. The major biochemical pathway for the metabolism of acrolein is conjugation with glutathione[4, 5]. Although because of its high reactivity acrolein reacts spontaneously with glutathione, the formation of Michael adducts between glutathione and acrolein is catalyzed by glutathione *S*-transferases (GSTs). Multiple GSTs catalyze the conjugation of glutathione with unsaturated aldehydes; however, glutathione *S*-transferase P (GSTP) displays the highest catalytic activity with small unsaturated aldehydes such as base propenals and acrolein[6]. Nonetheless, the role of GSTP in the *in vivo* metabolism of acrolein has not been studied, and it remains unclear whether GSTP regulates the urotoxic effects of acrolein generated during therapy. Others previous studies indicate that acrolein imparts a great inflammatory response characterized by sub-epithelial edema, neutrophil infiltration, hemorrhage and necrosis. Some investigators have demonstrated increases in pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1ß (IL-β) and type 2 cyclooxygenase within the bladder epithelium in response to CP therapy[6, 7]. Other factors such as cytokines, cyclooxygenase-2 (COX-2), and reactive oxygen species with other inflammatory mediators may contribute to the pathogenesis of CP-induced urotoxicity. Nitric oxide (NO) is suggested to play a significant role in the urinary tract injury associated with CP treatment[8, 9]. Oxidative stress damage manifested by a significant increase in the levels of malondialdehyde and inhibition of the peroxidase and catalase enzymes presents another major cause of the tissue damage[10, 11]. CP-induced urotoxicity is the major limiting step in its clinical application despite of its potent antitumor activity[12]. CP treatment also results in the production of reactive oxygen species (ROS), which cause peroxidative damage to urinary bladder and other vital organs[13]. Role of tissue anti-oxidants becomes important in the prevention of such damage. There is an overall decrease in the glutathione content in various tissues as a result of CP treatment[14]. In urinary bladder also the reduction in the levels of GSH (reduced glutathione) resulting from CP treatment has been reported[14]. The reactive metabolites of CP are responsible for various toxic as well as therapeutic actions of CP[1].

A number of natural products and synthetic compounds have been shown to reduce CP toxicity mainly due to their anti-oxidant action[14, 15]. *Rosmarinus tomentosus* is a vegetal species closely related to the culinary rosemary (*R. officinalis*), a plant reported to contain anti-hepatotoxic agents. In the present study, a dried ethanol extract of the aerial parts of *Rosmarinus tomentosus* (Rt) were evaluated in the restoration of anti-oxidants and reduction of lipid peroxidation (LPO) in urinary bladder in CP-treated animals which are predisposed or commitantly exposed to a known GSH-depleting agent (buthionine sulfoximine, BSO) alone and in combination with CP.

MATERIALS AND METHODS

Plant extract (PE)

Aqueous total extract of (*Rosmarinus tomentosus*) in semisolid form was purchased from the Plant Extract Division of the local Central Pharmacy, Tunisia. The moisture and ash content of extract were 12% and 8%, respectively and pH of 10% aqueous solution of extract was 4.6. Authenticity of extract was certified by the expert taxonomist of the manufacturers.

Chemicals

Cyclophosphamide monohydrate (2-(bis-(2-chloroethyl) amino) tetrahydro- 2H-1,3,2-oxazaphosphorine 2-oxide monohydrate); CAS 6055-19-2 and BSO (L-buthionine-SR-sulfoximine); CAS 5072-26-4, were purchased from Sigma–Aldrich Co., St. Louis, MO, USA.

Animals

The study was conducted in male Swiss albino mice (25 ± 2 g) provided by the animal service of Institut Pateur de Tunis, Tunisia. The study was approved by the Institutional Animal Ethics Committee (IAEC). The animals were bred and maintained under the standard laboratory conditions (temperature 25 ± 2 °C; photoperiod of 12 h). Commercial pellet diet and water

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were given *ad libitum*.

Dosage and experimental groups

BSO, CP and plant extract of *Rosmarinus tomentosus* (Rt) were suspended in normal saline. Animals were divided in seven groups, Group I–VII. Each group consisted of a minimum of six animals. Control animals (Group I) were administered normal saline p.o. for 10 days and a single i.p. injection of the same on the 10 th day. BSO (500 mg/kg body wt) was administered i.p. on the 10th day (Group II). CP (50 mg/kg body wt.) was given as a single i.p. dose on the 10th day of treatment (Group III). Group IV (BSO + CP) animals were administered BSO i.p. 5 h before CP administration. Group V (CP + Rt) animals were administered plant extract for 10 days along with a single i.p. injection of CP on 10th day. Group VI (BSO + Rt) animals were given plant extract treatment (100 mg/kg body wt.) p.o. for 10 days and a single i.p. injection of BSO on the 10th day along with the extract. Group VII (BSO + CP + Rt) animals were administered plant extract for 10 days and CP and BSO on the 10th day. Dosing was done in such a manner that all the animals could be sacrificed on the same day, i.e., day 11. Selection of doses of BSO and CP are based on pilot experiments using a range of doses and also taking into account previously published report[14, 16].

Biochemical investigations

After termination of treatment, animals were sacrificed under mild anesthesia and their bladders were removed. The bladder tissue was homogenized in chilled phosphate buffer (0.1 M, pH 7.4), using a Potter homogenizer. The homogenate was centrifuged at 10,500g for 30 min at 4 °C to obtain the post-mitochondrial supernatant (PMS), which was used for the biochemical measurements as described below.

Lipid peroxidation

LPO was measured using the procedure of Uchiyama and Mihara[36]. The assay mixture consisted of 0.67% thiobarbituric acid, TBA (Sigma-Aldrich), 10 mM butylated hydroxy toluene, BHT (Sigma-Aldrich), 1% ortho-phosphoric acid (Sigma-Aldrich) and tissue homogenate in a total volume of 3 ml. The rate of LPO was expressed as nmol of TBA reactive substances (TBARS) formed/h/g of tissue using molecular extinction coefficient (º) of 1.56×10^5 M^{-1} cm^{-1}.

Measurement of GSH

GSH content was measured in the PMS of urinary bladder by the method of Haque *et al.*[14]. PMS (1ml) was precipitated with 1ml of 4% sulfosalicylic acid (Sigma-Aldrich). The samples were incubated at 4 °C for 1 h and then centrifuged at 1200g for 15 min at 4 °C. The assay mixture consisted of 0.2 ml of filtered aliquot, 2.6 ml of sodium phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 100 mM DTNB (dithio-bis-2-nitrobenzoic acid, Sigma-Aldrich) in a total volume of 3 ml. The absorbance of reaction product was measured at 412 nm and results expressed as nmol GSH/g tissue.

Anti-oxidant enzyme measurements

Glutathione-S-transferase (GST) activity was assayed using the method of Haque *et al.*[14]. The reaction mixture consisted of 1.675 ml sodium phosphate buffer, 0.2 ml of 1mM GSH (Sigma-Aldrich), 0.025 ml of 1mM CDNB (1-chloro-2,4-dinitrobenzene, Sigma-Aldrich) and 0.1ml of PMS in a total volume of 2ml. The change in absorbance was recorded at 340 nm and the enzyme activity calculated as nmol CDNB conjugates formed/min/mg protein using º of 9.6×10^3 M^{-1} cm^{-1}. GR (glutathione reductase) activity was assayed by the method of Sharma et al.[17]. The assay mixture consisted of 1.6 ml sodium phosphate buffer, 0.1ml of 1mM ethylenediamine tetra acetic acid disodium salt (EDTA, Sigma-Aldrich), 0.1 ml NADPH (nicotinamide adenine dinucleotide phosphate reduced, Sigma-Aldrich) and 0.1ml oxidized glutathione (Sigma-Aldrich) and PMS (0.1ml) in total volume of 2ml. The enzyme activity measured at 340 nm was calculated as nmol NADPH oxidized/min/mg of protein, using º of 6.22×10^3 M^{-1} cm^{-1}. Glutathione peroxidase (GP) activity was assayed using the method of Sharma et al.[17]. The assay mixture consisted of 1.49 ml sodium phosphate buffer, 0.1ml EDTA (1mM), 0.1 ml sodium azide (1 mM, Central Pharmacy of Tunis, Tunisia), 0.1 ml of 1 mM GSH (Sigma–Aldrich), 0.1 ml NADPH (0.02 mM), 0.01 ml of 0.25 mM hydrogen peroxide (H_2O_2, CDH Chemicals) and 0.1 ml PMS in a total volume of 2ml. Oxidation of NADPH was recorded spectrophotometrically at 340 nm. The enzyme activity was calculated as nmol NADPH oxidized/min/mg of protein, using º of 6.22×10^3 M^{-1} cm^{-1}. CAT (catalase)

activity was assayed using the method used previously[14]. The assay mixture consisted of 1.95 ml phosphate buffer, 1ml H_2O_2 (0.09 M) and 0.05 ml of PMS in a final volume of 3 ml. Change in absorbance was recorded kinetically at 240 nm. CAT activity was calculated in terms of nmol H_2O_2 consumed/min/mg protein.

Protein measurement

Protein was measured by the method of Lowry *et al.*[18].

Statistical analysis

Single factor one-way analysis of variance (ANOVA) was applied to determine significant differences in results of various groups. P values <0.05 were considered significant. Subsequently, Student-Newman–Keuls test was applied for analyzing the significance between different treatment groups. The values are expressed as mean ± SE.

RESULTS

No mortality and significant change in the body weight were observed in different groups of animals.

Lipid peroxidation (LPO)

BSO treatment resulted in a significant increase ($P < 0.01$) in LPO in bladder over control values (Figure 1). CP treatment also induced LPO significantly. The cumulative effect of BSO + CP resulted in significant increase in the LPO as compared to Group I (control). BSO + CP group (Group IV) showed significant ($P < 0.01$) increase in the levels of LPO when compared to CP (Group III) alone (Figure 1). Animals pre-treated with *Rosmarinus tomentosus* (Rt) and subsequently exposed to BSO (BSO + Rt) showed a significant ($P < 0.01$) reduction in LPO in bladder. PE treatment also significantly ($P < 0.05$) reduced levels of LPO in bladder when the data of CP + Rt group (Group V) was compared with that of CP group (Group II). As shown in Figure 1, when the data of LPO of BSO + CP group (Group IV) and BSO + CP + Rt treatment group were compared, a significant decrease ($P < 0.01$) was observed in case of later (Group VII).

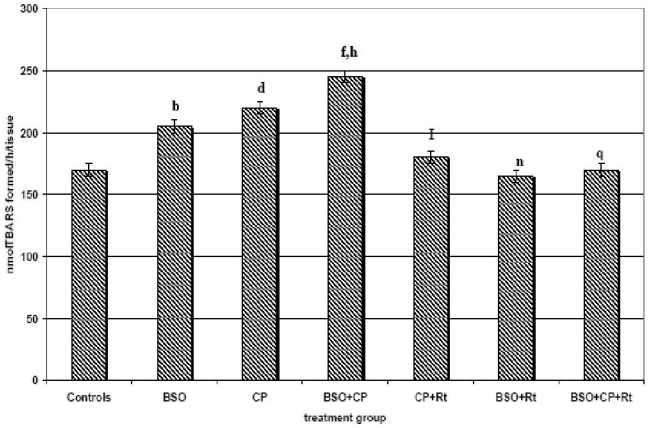

Figure 1 : Effect of *Rosmarinus tomentosus* (Rt), BSO and CP on the lipid peroxidation (LPO) in urinary bladder of mice. Significant differences are indicated by $^{b}P < 0.01$ and $^{d}P < 0.01$ in Group II (BSO) and Group III (CP) treated animals, respectively and $^{f}P < 0.01$ in Group IV (BSO + CP), when compared with control animals (Group I). $^{h}P < 0.01$ indicates significant levels of BSO + CP group when compared to CP group. $^{l}P < 0.01$ and $^{n}P < 0.05$ indicate significant difference of data of Group V (CP + Rt) and Group VI (BSO + Rt) when compared with Group III and Group II, respectively. $^{q}P < 0.01$ when Group IV data was compared with Group VII. Values are means ± SE (n = 6).

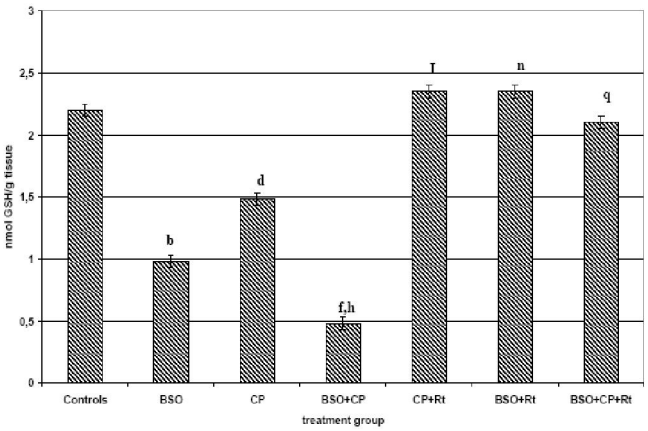

Figure 2 : Effect of *Rosmarinus tomentosus* (Rt), BSO and CP on GSH content in urinary bladder of mice. Significant differences are indicated by $^{b}P < 0.01$and $^{d}P < 0.01$ in Group II (BSO) and Group III (CP) animals, respectively and $^{f}P < 0.01$ in Group IV (BSO + CP) when compared with control animals (Group I). $^{h}P < 0.01$ indicates significant change in BSO+CP group when compared with CP group. $^{l}P < 0.01$ and $^{n}P < 0.01$ indicate significant differences in observations in Group IV (CP + Rt) and Group VI (BSO + Rt) when compared with GSH data of Groups III and II, respectively. $^{q}P < 0.01$ when GSH data of Group IV was compared with Group VII. Values are means ± SE (n = 6).

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Reduced glutathione

Significant (P < 0.01) decrease in GSH was observed in BSO, CP and BSO + CP-treated groups (2.5, 3.5 and 1.5 nmol GSH/g tissue, respectively) as compared to control values (5 nmol GSH/g tissue) (Cellular GSH data of urinary bladder are shown in Figure 2). Also, there was a significant (P < 0.01) decrease in the levels of GSH in BSO + CP group (Group IV) when compared to CP group (Group III). As shown in figure 2, when comparison was made between BSO and BSO + Rt groups, GSH content was found to be increased significantly (P < 0.01) in case of later. Similarly, bladder of CP + Rt group (Group V) showed significantly increased (P < 0.01) GSH content as compared to CP alone group (Group III). When the data of GSH of BSO + CP (Group IV) group was co mpared with BSO + CP + Rt group (Group VII), significant (P < 0.01) restoration in GSH was recorded (Figure 2).

TABLE 1 : Activities of anti-oxidant enzymes in the urinary bladder of mice in different treatment groups. Values are means ± SE (n = 6). GST expressed as nmol CDNB conjugates/ min/mg protein, GR as nmol NADPH oxidized/min/mg of protein, and GP as nmol NADPH oxidized/min/mg protein. CAT activity is expressed as nmol H_2O_2 consumed/min/mg protein. Significant differences are indicated by $^{b}P < 0.01$, $^{d}P < 0.01$, $^{c}P < 0.05$ and $^{f}P < 0.01$ when compared with control animals. (Group I) $^{n}P < 0.01$ when compared with Group II, $^{h}P < 0.01$ and $^{I}P < 0.01$ when compared with CP-treated animals (Group III) and $^{q}P < 0.01$ when compared with Group IV. Hamrita *et al.*, (Tab.1)

Group	Activity of anti-oxidant enzyme			
	GST	GR	GP	CAT
I (Controls)	105±3	104±4	116±5	65±3
II (BSO)	61±2 ^{b}	53±4^{b}	83±3^{b}	58±2^{b}
III (CP)	76±3^{d}	83±5 ^{d}	100±4^{c}	45±3^{d}
IV (BSO+CP)	40±3^{f,h}	47±2^{f,h}	79±3^{f,h}	43±3^{f}
V (CP+Rt)	114±5^{I}	113±5^{I}	123±2^{I}	68±5^{I}
VI (BSO+Rt)	108±3^{n}	110±6^{n}	117±4^{n}	74±7^{n}
VII (BSO+CP+Rt)	106±4^{q}	109±6^{q}	115±4^{q}	65±5^{q}

Anti-oxidant enzymes

BSO and CP treatments significantly (P < 0.01) decreased the activities of GST, GR, GP and CAT in the bladder when compared with control group (TABLE 1). BSO + CP group also showed an additive significant (P < 0.01) decrease in the activities of GST, GR and GP when compared with CP or control group. However, there was no significant difference in CAT activity between the animals of group IV (BSO + CP) and group III (CP). Activities of these anti-oxidant enzymes increased significantly (P < 0.01) in both BSO + Rt (Group VI) and CP + Rt (Group V) groups when compared to their respective controls, BSO (Group II) and CP (Group III) (TABLE 1). Animals treated with *Rosmarinus tomentosus* (Rt) and subsequently exposed to BSO + CP (Group VII, BSO + CP + Rt treatment) displayed a significant increase (P < 0.01) in the activities of all the anti-oxidant enzymes when compared with BSO + CP group (Group IV).

DISCUSSION

The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components[19]. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage and health problems[20, 21]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases[22]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources. These natural plant antioxidants can therefore serve as a type of preventive medicine. The antioxidant properties of the leaves phenolic compounds were reported in many studies: they act as a scavenger of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH). It was also shown that the presence of gallic acid and its derivative, the 1, 2, 3, 4, 6-pentagalloylglucose in the fruits, play a protecting role against lipid peroxidation induced by H_2O_2 in K562 cell line[23].

Currently, the research of natural antioxidants like alternative sources of synthesis antioxidants was

emerged and the exploitation of the various secondary metabolites of the plant was highlighted in recent years. In the same way, some researchers suggest that two-thirds of the world's plant species have medicinal value; in particular, many medicinal plants have great antioxidant potential. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. Different vegetal species have been described for providing active principles that protect against bladder damage in experimental animal models[24]. *Rosmarinus tomentosus*, a medicinal plant which has been previously reported for its antioxidatives effects in experimental animal models[25, 27]. This plant extract serves against the cold problems of the stomach, against reflux and vomiting. It is good for those who have spleen and liver problems. Furthermore, in form of a lavation it serves against all the cold problems of the head like epilepsy, stupor and somnolence. In the Mediterranean area, *Rosmarinus tomentosus* has been largely cultivated and numerous cultivars varying in flower colour and growth habits, etc., have been selected for their ornamental value. It is also an important source of essential oils and volatile compounds which are highly appreciated in both perfumery and pharmaceutical industries[26].

In cancer therapy, many medicinal plant extract have been demonstrated ameliorating toxicity induced by some drugs used in chemotherapy[27, 28]. CP and BSO-induced toxicity is the major limiting step in its clinical application despite of its potent antitumor activity. However, in bladder cancer, urotoxic effects of CP can be dose limiting and have proven fatal. Extensive hydration of patients receiving CP may alleviate urotoxicity; however, this treatment modality has been criticized as being difficult to properly maintain and may provide only partial protection from urinary bladder damage[29, 30]. Since bladder is the site for the storage of urine, concentration of toxic metabolites of CP is higher in bladder as compared to other organs, which increases the toxic load of CP in bladder to manifold. Moreover, the mechanisms that determine individual susceptibility to CP and mediate their bladder toxicity remain unclear. CP caused a significant reduction in the levels of all the anti-oxidants in urinary bladder. Besides, it increased LPO in the bladder. The oxidative products of the CP responsible for induction of LPO and generation of the ROS result in inflammation thus disturbing the overall redox cycling of the bladder[31]. Treatment with buthionine sulfoximine (BSO), a potent inhibitor of r-glutamylcysteine synthetase, has been reported to enhance the *in vitro* antitumor efficacy of a number of drugs including CP, melphalan, adriamycin, daunomycin and mitomycin C[32]. By inhibiting this essential enzyme, BSO has the capacity to drastically reduce glutathione content and has been shown in model systems to enhance the cytotoxic effects of specific chemotherapeutic agents and radiation therapy[33]. BSO and other agents directed at modulating glutathione levels affect the content of this metabolite in normal tissues as well as in tumors[34].

In the present work, *Rosmarinus tomentosus* extract has been used to examine its effect on CP and BSO-induced urotoxicity in mice. When data of reduction of GSH were compared, it was found that BSO has more significant effect than CP. A difference of 42% was noticed between the two observations confirming that BSO is a more potent depletor of GSH than CP as reported elsewhere[35]. CP-induced depletion of GSH is primarily mediated by interaction of its reactive metabolite, acrolein with GSH[5]. Acrolein not only interacts with GSH but also with cysteine which is one of the constituent amino acids of GSH[5]. Several reports in the literature have indicated that compounds containing free sulfhydryl groups may protect from the urotoxic effects of cyclophosphamide. A number of sulfhydryl (-SH) compounds and cysteine itself have been observed to protect the animals from toxic effects of CP[35].

Intraperitoneal administration of CP induced LPO in bladder in a significant manner. Lipid peroxidation is widely used as an indicator to reflect oxidative stress and cell membrane damage. Free radicals like superoxide anion and hydroxyl radical exert their toxic effect by acting on DNA, membrane proteins and lipids. CP-induced LPO has been reported in different tissues of exposed animals[36]. In CP-induced LPO also role of acrolein has been implied[37].

It has been suggested that by binding to nucleophilic amino acids, acrolein could directly affect transcription as well as modulate this process through its ability to deplete GSH[5]. BSO treatment resulted in depletion of

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GSH and increase in LPO in urinary bladder. Depletion of GSH is also reported to increase susceptibility of cells to apoptosis[38]. A depletion of intracellular GSH has been described in a number of different apoptotic systems, with several studies showing that GSH loss in cells undergoing apoptosis is the result of accelerated efflux rather than depletion by oxidation[39]. When BSO and CP were administered together, an additive effect was observed in case of GSH, LPO and other parameters. The purpose of using BSO along with CP was to study a likely scenario where host is exposed to a combination of GSH depleting agents including pathogens and to assess whether herbal extract treatment of *Rosmarinus tomentosus* has any modulatory effect on their commutative/additive effect. *Rosmarinus tomentosus* extract pre-treatment not only showed protective effect on CP urotoxicity but was also effective in protecting the animals treated with CP + BSO combination (Group VII). While herbal extract pre-treatment restored depleted GSH and other anti-oxidants, simultaneously it reduced LPO in the bladder. CP-induced immuno-suppression is likely to increase incidence of infections which may deplete GSH as many infectious agents are reported to deplete GSH[40]. An herbal extract like *Rosmarinus tomentosus* which has GSH restoring effect holds great promise in reducing the adverse effects of CP in cancer patients. A number of herbal extracts and their isolated constituents have also shown protective effect against CP-induced urotoxicity[41]. Thiols containing compounds such as mesna and cysteine have shown protective effects against CP-induced urotoxicity. *Rosmarinus tomentosus* extract has been extensively used in a number of herbal formulations. For centuries, it is being used as a folklore medicine. Its various pharmacological actions reported hitherto in ancient medicine literature have been scientifically validated later. In this context, Milagros Galisteo et al.[42] indicate that *Rosmarinus tomentosus* is a plant reported to contain anti-hepatotoxic agents suggesting the need to isolate the chemical principles responsible for this activity and to study this activity in a model of thioacetamide-induced cirrhosis. The findings of the present investigation demonstrate that *Rosmarinus tomentosus* extract pre-treatment prevented CP urotoxicity which is primarily mediated by LPO and depleted GSH by reversing these effects. Moreover, because of its widespread availability and relative lack of toxicity, *Rosmarinus tomentosus* deserves consideration for use in cancer patients as a complementary therapy after further validation and trials. In conclusion, the strongest evidence produced by this study supports an interaction between CP-induced urotoxicity and *Rosmarinus tomentosus* treatment.

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REFERENCES

[1] R.E.Fleming; Pharmacotherapy., **17**, 1465-45 **(1997)**.
[2] Baumann,R.Preiss; J.Chromatogr.B.Biomed. Sci.Appl., **764**, 173-192 **(2001)**.
[3] J.M.Patel, E.R.Block; Exp.Lung.Res., **8**, 153-165 **(1985)**.
[4] J.A.Cooper, W.W.Merrill, H.Y.Reynolds; Am.Rv.Respir.Dis., **134**, 108-114 **(1986)**.
[5] J.P.Kehrer, S.S.Biswal; Toxicol.Sci., **57**, 6-15 **(2000)**.
[6] V.Y.Hu, S.Malley, A.Dattilio, J.B.Folsom, P.Zvara, M.A.Vizzard; Am. J. Physiol. Regul. Integr. Comp.Physiol., **284**, 574-85 **(2003)**.
[7] R.A.Ribeiro, H.C.Freitas, M.C.Campos, C.C.Santos, F.C.Figueiredo, G.A.Brito; J.Urol., **167**, 2229-34 **(2002)**.
[8] F.S.Philips, S.S.Sternberg, A.P.Cronin, P.M.Vidal; Cancer.Res., **21**, 1577-1589 **(1961)**.
[9] A.B.Alfieri, L.X.Cubeddu; J.Pharmacol.Exper.Ther., **295**, 824-829 **(2000)**.
[10] L.H.Fraiser, J.P.Kehrer; Toxicology., **7**, 255-272 **(1992)**.
[11] Lee.FYF; Br.J.Cancer., **63**, 45-50 **(1991)**.
[12] J.G.Hengstler, A.Hengst, J.Fuchs, B.Tanner, J.Phol, F.Oesch; Mutat.Res., **373**, 215-223 **(1997)**.
[13] J.M.Patel; Toxicology., **45**, 79-91 **(1987)**.
[14] R.Haque, B.Bin-Hafeez, S.Parvez, S.Pandey, I.Sayeed, M.Ali; Hum.Exp.Toxicol., **22**, 473-480 **(2003)**.
[15] A.R.Abd-Allah, A.M.Gado, A.A.Al-Majed, A.A.Al-Yahya, O.A.Al-Shabanah; Clin.Exp. Pharmacol. Physiol., **32**, 167-172 **(2005)**.
[16] M.Ishikawa, Y.Takayanagi, K.Sasaki; Res.Commun. Chem.Pathol.Pharmacol., **65**, 265-268 **(1989)**.
[17] N.Sharma, P.Trikha, M.Athar, S.Raisuddin; Mutat.Res., **481**, 179-188 **(2001)**.

[18] O.Lowry, N.J.Rosebrough, R.J.Randall; J.Biol.Chem., **193**, 265-275 **(1951)**.
[19] J.C.Winston; Am.J.Clin.Nutr., **70**, 491-499 **(1999)**.
[20] E.Peuchant, J.Brun, V.Rigalleau, L.Dubourg, M.Thomas, J.Daniel; Clin.Biochem., **37**, 293-298 **(2004)**.
[21] P.Steer, J.Milligard, D.M.Sarabi, B.Wessby, T.Kahan; Lipids., **37**, 231-236 **(2002)**.
[22] V.Di-Matteo, E.Esposito; Curr.Drug.Targets. CNS.Neurol.Disord., **2**, 95-107 **(2003)**.
[23] A.Abdelwahed, I.Bouhlel, I.Skandrani, K.Valenti, M.Kadri, P.Guiraud; Chem.Biol.Interact., **165**, 1-13 **(2007)**.
[24] K.Bhatiaa, M.Kaura, F.Atifa, M.Alia, H.Rehmana, S.Rahmana; Food.And Chemical Toxicology., **44**, 1744-1750 **(2006)**.
[25] B.Bozin, N.Mlmica-Dukic, I.Samojlik, E.Jovin; J.Agr.Food.Chem., **55**, 7879-7885 **(2007)**.
[26] M.C.Navarro, M.P.Montilla, A.Martýn, J.Jimenez, M.P.Utrilla; Planta Med., **59**, 312-314 **(1993)**.
[27] N.D.Scott; Altern.Med.Rev., **4**, 178-189 **(1999)**.
[28] S.S.Ali, N.Kasoju, A.Luthra, A.Singh, H.Sharanabasava, A.Sahu; Food Res.Int., **41**, 1-15 **(2008)**.
[29] J.A.Botta, L.W.Nelson, J.H.Weikel; J.Nati.Cancer Inst., **51**, 1051-1057 **(1973)**.
[30] M.A.Colvin; Clinical. Pharmacology of Anti-Neoplastic Drugs., 245-261 **(1978)**.
[31] J.A.Cooper, W.W.Merrill, H.Y.Reynolds; Am.Rv.Respir.Dis., **134**, 108-114 **(1986)**.
[32] R.F.Owls, K.O.Louie, J.Plowman, B.C.Behrens, R.L.Fine, D.Dykes; Biochem.Pharmacol., **36**, 147-1531 **(1987)**.
[33] J.E.Biaglow, M.E.Varnes, E.R.Epp, E.P.Clark, S.W.Tuttle, K.D.Held; Int.J.Radiat.Oncol.Biol.Phys., **16**, 1311-1311 **(1989)**.
[34] A.Meister; Mechanisms.of Drug Resistance In Neoplast.Cells., 99-126 **(1988)**.
[35] M.Ishikawa, Y.Takayanagi, K.Sasaki; Res.Commun.Chem.Pathol.Pharmacol., **65**, 265-268 **(1989)**.
[36] E.Selvakumar, C.Prahalathan, Y.Mythili, P.Varalakshmi; Mol.Cell.Biochem., **272**, 179-185 **(2005)**.
[37] J.D.Adams, L.K.Klaidrnan; Free.Radic.Biol.Med., **15**, 187-193 **(1993)**.
[38] N.Mirkovic, D.W.Voehringer, M.D.Story, D.J.McConkey, T.J.McDonell, R.E.Meyn; Oncogene., **15**, 1461-1470 **(1997)**.
[39] L.Ghibelli, C.Fanelli, G.Rotilio, E.Lafavia, S.Coppola, C.Colussi; FASEB.J., **12**, 479-486 **(1998)**.
[40] C.R.Hung, P.S.Wang; Eur.J.Pharmacol., **491**, 61-68 **(2004)**.
[41] L.Davis, G.Kuttan; Cancer Lett., **148**, 9-17 **(2000)**.
[42] M.Galisteo, A.Suarez, M.P.Montilla, M.P.Utrilla, J.Jimenez, A.Gil; Phytother.Res., **14**, 522-526 **(2000)**.