The chemopreventive effect of dimethylthiourea against carmustine-induced myelotoxicity in rats

El-Sayed M. El-Sayed a,*, Abdel-Aziz H. Abdel-Aziz a, Samira Saleh b, Ahmed S. Saad a

aPharmacology and Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
bPharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

A R T I C L E   I N F O

Article info
Received 13 November 2010
Accepted 6 May 2011
Available online 19 May 2011

Keywords:
Carmustine
Dimethylthiourea
Myelosuppression

A B S T R A C T

The possible chemopreventive role of dimethylthiourea (DMTU) against carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea, BCNU)-induced myelotoxicity was assessed through evaluation of apoptosis, lipid peroxidation, glutathione (GSH) content and some antioxidant enzymes activities in bone marrow cells of rats. Thirty-six rats were randomly classified into four groups. The first group was injected i.p. with ethanol and served as a control. The second group was treated with BCNU. The third group was given DMTU, while the fourth group was co-administered with DMTU prior to BCNU administration. BCNU treatment in a single dose of 30 mg/kg significantly decreased the normal counts of RBCs, WBCs and platelets as well as hemoglobin level. In addition, BCNU exhibited marked apoptotic effect associated with significant alterations in the oxidative cascade parameters. Treatment of animals with DMTU in a single dose of 500 mg/kg 1 h before BCNU injection, followed by 125 mg/kg twice daily for 5 consecutive days significantly mitigated the induced changes in the hematological parameters. The induced alterations in the oxidant and antioxidant parameters as well as apoptosis were also improved. Conclusively, DMTU treatment exhibited marked chemopreventive effect against BCNU-induced myelotoxicity; an effect which may be partially attributed to its inherently antioxidant potential.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Myelosuppression is one of the most common dose-limiting toxicity in cancer treatment (Moritz et al., 1995; Maze et al., 1996; Volpe and Warren, 2003). The extreme sensitivity of bone marrow to BCNU may be explained by the inability of this tissue to repair BCNU-induced cytotoxic DNA damage efficiently (Gerson et al., 1986; Maze et al., 1996). BCNU can produce cross-linking by different mechanisms (Kohn, 1977; Tong et al., 1982) mainly in two steps: chloroethylation of a nucleophilic site on one strand and displacement of a chloride ion on the other strand which results in formation of ethyl bridge between the strands that prevents unwinding of the DNA double helix. The alkylating moiety is further decomposed to the carbonium ion and reacts in the cell to form DNA monoadducts and cross-linked DNA. The carbamoylating isocyanate moiety also reacts with the –SH, –OH, and –NH₂ groups of proteins and causes enzyme inactivation (Brundrett, 1980).

Extensive carbamoylation of proteins by BCNU in vitro results in inactivation of some proteins, such as chymotrypsin, alcohol dehydrogenase and glutathione reductase (Frischer et al., 1993). Previous studies revealed that, glutathione reductase (GR), a NADPH-dependent antioxidant enzyme, is massively inhibited by BCNU treatment (Helal and Helal, 2009). It has been also found that chemotherapeutic agents, including BCNU can induce cellular oxidative stress-mediated apoptosis (Fanger et al., 1997; Ip and Davis, 1998; Helal and Helal, 2009). The presumed explanation by which BCNU can induce apoptosis is mainly related to increased oxidative stress following carbamoylation of tissue cysteines (Ahmad and Frischer, 1985).

DMTU is a water-soluble synthetic thiol antioxidant (Milner et al., 1993; Roberts et al., 1996; Sprong et al., 1997) that is an effective scavenger of toxic reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, and hypochlorous acid (Fox, 1984; Curtis et al., 1988). Its protective effect has been described in different tissues and pathologies (Roychoudhury et al., 1996; Baek et al., 2003; Tsuruya et al., 2003). It can reduce ocular injury associated with uveitis and endotoxin treatment (Rao et al., 1988; Fleischer et al., 1989), granulocyte-mediated lung injury (Fox, 1984; Huang et al., 2002) and reperfusion-induced damage to the brain (Patt et al., 1988). Also, DMTU has a protective effect in cyclic light and dark-reared rats exposed to intense visible light (Organisciak et al., 1992). In addition, DMTU was found to be effective in treatment of neuropathy and vasculopathy in diabetic rats (Cameron et al., 2001). Moreover, it was reported to attenuate the cardiac dysfunction contractility and tissue injury (Pattanaik and Prasad,
2001), prevent hepatic cirrhosis in rats (Bruck et al., 2001) and protect against mitochondrial oxidative damage induced by cisplatin in liver of rats (Santos et al., 2007). Furthermore, it was documented to ameliorate cisplatin-induced nephrototoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria (Santos et al., 2008). However, the effectiveness of DMTU in treatment of BCNU-induced-myelotoxicity is still not fully explored. Therefore, the present work was designed to study the extent of bone marrow toxicity after BCNU administration as well as the possible chemopreventive effect of DMTU against such toxicity.

2. Materials and methods

2.1. Animals

Male Wistar albino rats weighing 110–130 g were obtained from National Institute of Cancer, Cairo and housed in the animal facility. Pharmacology and Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt for 15 days before the experimentation. Animals were fed standard diet pellets (El-Nasr Co., Ahou-Zaabal, Egypt) and maintained under 12 h light/12 h dark cycle at a constant temperature (22 ± 2 °C), with water ad libitum.

2.2. Chemicals

Carmustine (BCNU) was purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA), dissolved in ethanol 95% and injected i.p. in a single dose of 30 mg/kg bwt in a volume not exceeding 0.1 ml/100 g rat. This dose was comparable to the human therapeutic dose in the treatment of brain tumor (Chu and Sartorelli, 2007) based on body surface area conversion ratios (Paget and Barnes, 1964). DMTU was purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA), dissolved in distilled water and injected i.p. a single dose of 500 mg/kg bwt 1 h before BCNU injection, followed by 125 mg/kg bwt twice a day for 5 days (Tsruya et al., 2003). All other chemicals were of the highest available commercial grade.

2.3. Experimental design

In the present study 36 animals were randomly classified into four groups (each of 9) as follows:

Group I: injected i.p. with ethanol 95% in a dose of 0.1 ml/100 g rat and served as control group.

Group II: injected i.p. with BCNU in a single dose of 30 mg/kg bwt.

Group III: injected i.p. with DMTU in a single dose of 500 mg/kg bwt for one day followed by injection with 125 mg/kg bwt twice a day for the next 5 consecutive days.

Group IV: injected i.p. with DMTU in a single dose of 500 mg/kg bwt 1 h before a single i.p. dose of BCNU (30 mg/kg bwt), followed by injection with 125 mg/kg bwt twice a day for the next 5 consecutive days.

The animals were killed by cervical dislocation one week post BCNU administration. Three animals from each group were used for apototic study in bone marrow cells and the remaining 6 rats were used for biochemical and hematological analyzes. Blood samples were collected and femurs were dissected out where bone marrow was excised out from each animal on ice.

2.4. Collection of blood samples

Venous blood was obtained using siliconized capillary tubes to puncture the orbital sinus of rats (Riley, 1960; Sorg and Buckner, 1964), where red blood cells (RBCs), white blood cells (WBCs) and platelets counts as well as hemoglobin (Hb) level were assessed for each sample in each group.

2.5. Preparation of bone marrow cells

Bone marrow cells suspension was prepared from femurs of each animal, where the bone is split longitudinally and the marrow is exposed using forceps and the content of the femur is flushed gently using 2 ml syringe containing 1 ml of DMEM media into a collection tube. The cell suspension was centrifuged and washed three times with DMEM media. The final suspension was used for evaluation of apoptosis (using free cell suspension), extent of lipid peroxidation (by measuring malondialdehyde (MDA) content), reduced glutathione (GSH) content and the activities of antioxidant enzymes including glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) (in the cell lysate) (Bolliger, 2004).

2.6. Determination of bone marrow cells apoptosis

Apoptosis was evaluated by flowcytometric analysis using Apoptarget kit (Dako–Cytomation, Denmark). Bone marrow cells were washed with phosphate buffer solution (PBS) and pooled with floating cells. Annexin-V binding buffer was diluted 1:10 in distilled water. Cells with PBS were washed twice and resuspended to 2–3 × 10^6 cells/ml. Aliquots were taken from cells at 100 μL/tube. Five μl of annexin-V and 10 μL of propidium iodide buffer were added to each tube and the tubes were incubated at room temperature for 15 min in the dark. Four-hundred μl annexin-V binding buffer were added to each tube and submitted to flowcytometric analysis.

2.7. Preparation of cell lysate

Cell lysate from bone marrow cells was prepared as described by Attica et al. (2009). Bone marrow cells were washed three times by DMEM media, and centrifuged for 10 min at 1000 rpm to remove the supernatant. The cells pellet was lysed by three repetitive freezing/thawing cycles, followed by homogenization of the cells by passing through a 20G needle and centrifugation at 2000g. Two μl of the supernatant (cell lysate) were stored to measure the protein content by the bicinchoninic acid (BCA) assay.

2.8. Determination of protein content (Smith et al., 1985)

Protein content was evaluated adopting BCA assay. This assay is efficient for detecting small amounts of protein (0.5–7.5 μg) using bovine serum albumin as a standard where the measurement was done at 560 nm using microplate reader.

2.9. Determination of MDA as a marker of lipid peroxidation

Peroxidative damage to cellular lipid constituents was determined by measuring the malondialdehyde (MDA) concentration in butanol extracts according to Uchiyama and Miura (1978) with some modifications (Leeuwenburgh et al., 1994). Briefly, 10 mmol/l butylated hydroxytoluene and 200 mmol/l ferrous sulfate were included in the assay mixture. Sealed tubes were incubated for 15 min at 99 °C. MDA concentration was calculated based on a standard curve using 1,1,3,3-tetraethoxyxpropane (malondialdehyde diethyl acetal, Sigma–Aldrich Corp., St. Louis, MO, USA) as a standard.

2.10. Determination of glutathione content

Total glutathione concentrations were determined according to the method of Griffith (1980), where 0.1 ml of the cell lysate was added to the reaction mixture (0.5 ml of 200 mM sodium phosphate buffer (pH 7.5), 0.1 ml of 50 mM Na2EDTA, 0.1 ml of 2 mM N,N-DPNH, 0.1 ml of 6 mM dithionitrobenzoic acid in 0.2 M sodium phosphate buffer (pH 7.5), and 0.1 ml of 0.5 unit/ml glutathione reductase (Sigma, MO, USA)), and then the reaction was measured at 412 nm for 3 min under 30 °C. After the removal of reduced GSH by adding 2 μl of 1 M 2-mercaptoethanol in 0.1 ml of the cell lysate and incubation at 25 °C for 1 h, the oxidized glutathione concentrations were determined as described above. A standard curve was prepared based on 0–20 nmol oxidized glutathione (Sigma, MO, USA). The reduced glutathione (GSH) concentrations were calculated by the subtraction of oxidized glutathione concentrations from total glutathione concentrations.

2.11. Estimation of antioxidant enzymes activity

2.11.1. Glutathione peroxidase (GPx) activity

GPx activity was determined following the method of Lawrence and Burk (1976) by quantifying the rate of oxidation of GSH by cumene hydroperoxide (Sigma–Aldrich Corp., St. Louis, MO, USA) as catalyzed by GPx present in the cell lysate samples. In a 96 well-microplate, 25 μl of cell lysates were incubated with 175 μl of buffer (100 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA, 1 mM NaN3, 1 mM GSH, 1 mM glutathione reductase) for 10 min. NADPH (25 μl of 1.5 mM) was added and the mixture was incubated for 10 min. The reaction was initiated by the addition of 25 μl of 0.8 mM cumene hydroperoxide, and measured at 340 nm for 10 min (30 s intervals).

2.11.2. Catalase activity (CAT)

CAT activity was assessed in the cell lysate adopting the method of Aebi (1983). The principle of the assay is based on the decomposition of H2O2 which was detected by the decrease in absorbance at 240 nm. The absorbance of the reaction mixture was recorded every 1 min for 5 continuous min using deionized water as a blank.
2.11.3. Superoxide dismutase (SOD) activity

The SOD activity was estimated in the cell lysate according to the method of Ewing and Janero (1995) using the nitro blue tetrazolium/phenazine methosulfate (NBT/PMS) assay. Assessment of superoxide dismutase activity was performed at a wavelength of 595 nm.

2.11.4. Statistical analysis of data

Data were statistically analyzed using computerized Instat prism 4 program. Data in Table 1 showed that BCNU treatment significantly decreases RBCs, WBCs and platelets counts as well as hemoglobin level amounting to 30%, 58%, 69%, and 24%, respectively as compared with the corresponding control ethanol-treated group. On the other hand, DMTU treatment significantly increased RBCs, WBCs and platelets counts as well as hemoglobin level recording 33%, 74%, 220%, and 30%, respectively in comparison with BNCU-treated group.

Data in Table 2 revealed that injection of rats with ethanol, i.p., produced bone marrow apoptosis amounting to 4%. However, treatment with BCNU in a single dose of 30 mg/kg, i.p., significantly increased the bone marrow apoptosis recording 21%. On the other hand, treatment of animals with DMTU in a single dose of 500 mg/kg bwt 1h before BCNU injection, followed by 125 mg/kg bwt twice a day for 5 days, significantly decreased% apoptosis in BCNU-treated rats to reach the extent of 12%.

Data in Table 3 showed that BCNU treatment significantly increased the bone marrow content of MDA reaching 179% and significantly decreased the glutathione bone marrow content as well as the activities of Gpx, Cat, and Sod amounting to 52%, 43%, 19%, and 48%, respectively as compared with the control group. On the other hand, treatment of animals with DMTU significantly ameliorated the changes in such oxidant and antioxidant parameters induced by BCNU.

3. Results

Data in Table 1 showed that BCNU treatment significantly decreases RBCs, WBCs and platelets counts as well as hemoglobin level amounting to 30%, 58%, 69%, and 24%, respectively as compared with the corresponding control ethanol-treated group. On the other hand, DMTU treatment significantly increased RBCs, WBCs and platelets counts as well as hemoglobin level recording 33%, 74%, 220%, and 30%, respectively in comparison with BNCU-treated group.

Data in Table 2 revealed that injection of rats with ethanol, i.p., produced bone marrow apoptosis amounting to 4%. However, treatment with BCNU in a single dose of 30 mg/kg, i.p., significantly increased the bone marrow apoptosis recording 21%. On the other hand, treatment of animals with DMTU in a single dose of 500 mg/kg bwt 1h before BCNU injection, followed by 125 mg/kg bwt twice a day for 5 days, significantly decreased% apoptosis in BCNU-treated rats to reach the extent of 12%.

Data in Table 3 showed that BCNU treatment significantly increased the bone marrow content of MDA reaching 179% and significantly decreased the glutathione bone marrow content as well as the activities of Gpx, Cat, and Sod amounting to 52%, 43%, 19%, and 48%, respectively as compared with the control group. On the other hand, treatment of animals with DMTU significantly ameliorated the changes in such oxidant and antioxidant parameters induced by BCNU.

4. Discussion

Data in the present study revealed that injection of rats with BCNU in a single dose of 30 mg/kg, i.p., significantly increased bone marrow apoptosis and bone marrow content of MDA when compared with their corresponding control-ethanol-treated group, but decreased the bone marrow activities of SOD, Gpx and Cat as well as the glutathione bone marrow content. The obtained data are in line with previous studies indicating that BCNU chemotherapy down-regulates Bcl-xl and Bcl-2 resulting in enhancement of apoptosis (Lytte et al., 2004). Also, recent studies showed that BCNU is a glutathione depletor and is able to induce proinflammatory cytokines including TNF-α with subsequent induction of apoptosis (Helal and Helal, 2009). In fact, BCNU was found to induce an irreversible injury of glutathione-S-transferase (Institorios et al., 1993). In addition, the increased extent of apoptosis in the present work can be explained by the fact that bone marrow tissue has very low levels of O6-methylguanine DNA methyltransferase (O6-MeG DNA Mtase) and 3-methyladenine DNA glycosylase activities relative to other tissues (Gerson et al., 1986; Moritz et al., 1995). Growing evidences of studies indicated that imbalance in redox status by BCNU can substantially increase the permeability of plasma membrane to Ca2+ and Na+ leading to subsequent cell death (Koliwad et al., 1996; Herson et al., 1999; Harra et al., 2002; Mukherjee et al., 2002).

The reduced activities of Cat, SOD and Gpx in the present study may be explained by the fact that oxidative stress is a mediator of myelotoxicity (Maxwell, 1995). It has been stated that Gpx is capable of reducing free hydrogen peroxide where SOD and CAT provide the first defense against oxygen toxicity by catalyzing the dismutation of superoxide anion to hydrogen peroxide and decomposition of hydrogen peroxide to water and molecular oxygen, respectively (Sundaresan and Subramanian, 2003).

The decreased activity of the antioxidant enzymes Gpx in this work may be due to either the decreased level of GSH as a substrate (Vo et al., 1988) or inability of bone marrow cells to produce this enzyme. On the other hand, previous studies have reported that the activity of Gpx is strongly dependent upon the state of thiol system (Jayalakshmi et al., 2007). The decreased activity of bone marrow Gpx by BCNU is in consistence with Hagen et al. (1988) and Weydert et al. (2003) who stated that the reduction of Gpx enzyme activity after exposure to BCNU may be explained by a reduced capacity of this enzyme to scavenge free radicals.

Data in the present study showed that administration of BCNU resulted in reduction of RBCs, WBCs and platelets counts as well as hemoglobin level. These data are in agreement with previous studies indicating that BCNU has been one of hematotoxic agents (Pessina et al., 2003). The authors explained that anticancer therapy could lead to reversible perturbations in peripheral blood counts.

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>RBCs (10^6/mm^3)</th>
<th>WBCs (10^6/mm^3)</th>
<th>Platelets/mm^3</th>
<th>Hb (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ethanol)</td>
<td>7.29 ± 0.35</td>
<td>11.5 ± 1.07</td>
<td>695 ± 4.62</td>
<td>13.65 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>DMTU</td>
<td>6.93 ± 0.28</td>
<td>11.23 ± 0.27</td>
<td>762 ± 10.8</td>
<td>13.95 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>5.11 ± 0.43 ab</td>
<td>4.88 ± 0.34 ab</td>
<td>218 ± 2.03 ab</td>
<td>10.35 ± 0.91 ab</td>
<td></td>
</tr>
<tr>
<td>DMTU + BCNU</td>
<td>6.8 ± 0.28c</td>
<td>8.48 ± 0.5c</td>
<td>696 ± 51.3</td>
<td>13.46 ± 0.5c</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM (n = 6).

a Significantly different from control ethanol-treated group.

b Significantly different from DMTU-treated group.

c Significantly different from BCNU-treated group.

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Apoptosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ethanol)</td>
<td>3.70 ± 0.60</td>
</tr>
<tr>
<td>DMTU</td>
<td>3.36 ± 0.75</td>
</tr>
<tr>
<td>BCNU</td>
<td>21 ± 2.64b</td>
</tr>
<tr>
<td>DMTU + BCNU</td>
<td>11.45 ± 0.95ab</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SDM (n = 3).

a Significantly different from control ethanol-treated group.

b Significantly different from DMTU-treated group.

c Significantly different from BCNU-treated group at P < 0.05 using one way ANOVA test followed by Tukey-Kramer post-test for multiple comparison between groups.
Table 3
Effect of BCNU, DMTU and their combination on MDA and antioxidant parameters in bone marrow cells of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (nmole/mg protein)</th>
<th>GSH (nmole/mg protein)</th>
<th>GPx (mU/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ethanol)</td>
<td>14.20 ± 1.16</td>
<td>46.14 ± 2.30</td>
<td>5.59 ± 0.28</td>
<td>395.20 ± 10.6</td>
<td>5.10 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>DMTU</td>
<td>13.88 ± 1.24</td>
<td>42.72 ± 0.69</td>
<td>6.76 ± 0.22</td>
<td>490.40 ± 12.67</td>
<td>5.35 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>39.64 ± 2.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22.00 ± 2.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.19 ± 0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>321.55 ± 16.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.63 ± 0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DMTU + BCNU</td>
<td>21.28 ± 1.98&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>40.18 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.34 ± 0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>450.78 ± 16.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.15 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM (<i>n</i> = 6).

<sup>a</sup> Significantly different from control ethanol-treated group.

<sup>b</sup> Significantly different from DMTU-treated group.

Also, Parchment et al. (1998) reported that hematological toxicity (acute cytopenia or bone marrow suppression) is frequently encountered side effect of cytotoxic chemotherapy of cancer. In addition, Abushamaa et al. (2002) found that administration of BCNU as one component of breast cancer regimen resulted in decreased blood cell count after one and two weeks from administration. On the other hand, it could be explained that the oxidative stress in environment in the blood produced by BCNU may be implicated in the reduced RBCs, WBCs and platelets counts as well as the level of hemoglobin. This suggestion is augmented by previous studies suggesting that depletion of glutathione produces hematological toxicity and prevents enhancement of the therapeutic index of melphanan (Bailey et al., 1994; 1997; Alexandre et al., 2006).

On the other hand, treatment of animals with DMTU in a single dose of 500 mg/kg bwt twice a day for 5 days ameliorated the hematological, apoptotic, oxidant and antioxidant alterations in bone marrow induced by BCNU. DMTU, a thiol agent is a hydroxyl radical scavenger whose beneficial antioxidant effect against cytotoxicity has been explored in different tissues, but nevertheless, the exact mechanisms by which this action is exerted, especially those involving mitochondria, have not been fully elucidated. DMTU has been demonstrated to be highly effective in minimizing diabetic neurovascular complications, which are associated with increased oxidative stress (Cameron et al., 2001). According to Roychoudhury et al. (1996), pretreatment with DMTU prevented hydroxyl radical formation and reduced the lipid peroxidation induced by H<sub>2</sub>O<sub>2</sub> in mitochondria of pulmonary vascular smooth muscle tissue. DMTU has been shown to prevent cisplatin-induced acute renal failure in a mechanism associated with a decrease in apoptosis through mitochondrial pathways (Baek et al., 2003) and through death receptors pathways (Tsuruya et al., 2003). In conclusion, DMTU could have a potential protective effect against BCNU-induced myelotoxicity; an effect that is mainly attributed to either its free radical-scavenging effect due to its thiol content. DMTU could be beneficial as an adjuvant therapy to reduce myelosuppression for cancer patient under BCNU chemotherapy.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

### References


Mukherjee, S.B., Das, M., Sudhandiran, G., Shaha, C., 2002. Increase in cytosolic Ca\textsuperscript{2+}


