Mangiferin Attenuates Doxorubicin-Induced Nephrotoxicity in Rats Through Reduction of Oxidative Stress

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Abstract

Doxorubicin (DOX) has been widely used in the clinical setting for malignancy treatment. However, it also induces toxicity in a vital organ such as the kidney. Several studies suggest the formation of an iron anthracycline complex which produces free radicals plays an important role in nephrotoxicity.

Mangiferin (MGF), a xanthone derivative, exhibits as an iron chelator and antioxidant activity through a free radical scavenger activity. We analyzed the effect of MGF to prevent DOX induced nephrotoxicity. Male Sprague-Dawley rats were administered MGF orally every day for five weeks, with 50 and 100 mg/kg BW together with DOX. Renal failure and dyslipidemia were detected in the DOX group in association with increased malondialdehyde (MDA) levels in plasma and kidney and decreased superoxide dismutase (SOD) and glutathione (GSH) levels in kidney. Of note, co-treatment with MGF improved renal dysfunction caused by doxorubicin as shown by the amelioration plasma albumin, urea and creatinine levels, and proteinuria. MGF also diminished over-production of MDA levels, thus enhanced antioxidant activity such as SOD and GSH in the kidney.

Our study opens the perspective to clinical studies for consideration of MGF as a potential chemoprotectant nutraceutical in the combination chemotherapy with DOX to limit its nephrotoxicity.


Keywords: Doxorubicin, mangiferin, kidney, toxicity.

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Introduction

Doxorubicin (DOX) has been clinically used as one of the backbone anticancer drugs for solid and hematological malignancies. DOX exhibits its anticancer activity through inducing the inhibition of topoisomerase II enzyme which causes the accumulation of protein-linked double and single-strand DNA breaks (cleavage complex), thus consequently leading into cytotoxic DNA damage and cell death. However, DOX treatment has been considered to exhibit toxicity in multi-organ, including in the kidney. The molecular mechanism underlying DOX-induced toxicity is multi-factorial and, to date, not fully characterized yet, but the most acceptable theory attributes the initiation of such toxicity in the kidney is the oxidative stress accumulation.

We have previously reported that the progressive nephrotoxicity and cardiotoxicity after induction of daunorubicin (another anthracycline) is associated with the enhancement of oxidative stress, and apoptosis. Other studies also focused on antioxidants as the possible preventive supplementation drugs for overcoming the DOX toxicity, although the majority of reported studies were directed only in DOX cardiotoxic effects, and very few aiming at its renal toxicity.

Mangiferin (MGF), a natural compound of glucosyl xanthone which commonly used in several traditional medicinal plants, has been shown to produce multiple pharmacological effects which include antioxidant and anti-inflammatory and immunomodulatory activities. Previous study demonstrated that MGF has the iron complexing ability and protects against Fe2+-citrate induced lipid peroxidation. To the best of our knowledge, no study has
explored the protective effects of MGF against DOX-induced nephrotoxicity that have been published yet. The present study was conducted to identify the possible protective effect MGF administration against DOX induced nephrotoxicity through biochemical and oxidative stress approaches. Furthermore, the study hopefully might emphasize the use of evidence-based natural herbal medicine to reduce the toxicity of the chemo drug.

**Materials and methods**

**Drug and chemicals**

Unless otherwise stated, all reagents were analytical grade and purchased from Sigma-Aldrich- Merck Ltd, Jakarta, Indonesia. Assay kits for urea, creatinine, and lipid profile were obtained from DiaSys Diagnostic System GmBH (Jakarta, Indonesia). DOX was obtained as doxorubicin hydrochloride (2mg/ml) from Kalbe Pharma, Indonesia. MGF was analytical grade and purchased from Plamed Science Techno Company (Xian, China).

**Experimental animals**

Twenty-four adult male Sprague-Dawley rats (8-12 weeks and weighing 170-200 g) were obtained from Indonesia National Agency of Drug and Food Control, Jakarta, Indonesia. Animals were treated in accordance with the guide for the Care and Use of Laboratory Animals. They were housed under controlled environmental conditions of temperature (21°C), a relative humidity (55%) and a 12-h light/12-h dark cycle. They were allowed free access to standard laboratory food and water.

All experimental protocols were approved by the Animal Care Committee of Faculty of Medicine, University of Indonesia, Jakarta, Indonesia. The rats were randomly assigned into four groups, six animals each. After randomization into different experimental groups, the rats were acclimatized to the laboratory conditions for one week before beginning the experiment. Control group was intraperitoneally (i.p.) injected with vehicle (saline) and served as a normal control group (N). DOX group was injected with doxorubicin 2.5 mg/kg i.p in six equal injections at 48h intervals for two consecutive weeks to achieve a total dose of 15 mg/kg body weight (BW), which well documented to achieve nephrotoxicity, and served as a toxic control group. DOX+MGF group received DOX plus orally MGF with this following dose: 50 mg/kg BW (DOX+MGF50), and 100 mg/kg BW (DOX+MGF100).

Administration of MGF was started on the same day of DOX administration and continued for further three weeks after stopping of DOX (5 weeks total period). The doses and schedules of the study were slightly modified from our previous report. On day 34, rats were placed individually in metabolic cages to obtain 24-h urine collections for the measurement of protein concentrations. After the end of the study period (5 weeks), rats were sacrificed after measuring BW, blood was collected and kidney tissue was harvested for biochemical assays.

**Estimation of biochemical parameters**

Blood samples collected in heparinized syringes by heart puncture during sacrificed were utilized for the subsequent determination creatinine, albumin, total cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), triglyceride, and blood urea nitrogen (BUN) and was stored at -80°C. TC, LDL-C, albumin and triglyceride levels were determined using standard enzymatic procedures. Urinary protein excretion was determined by the Bradford method. Serum creatinine level was determined by the Jaffe method. BUN was determined by the diacetyl monoxime method.

**Determination of Lipid Peroxides**

Malondialdehyde (MDA), a reactive aldehyde for measuring lipid peroxidation, was analyzed in serum and kidney tissue according to the method of thiobarbituric acid reactive substance and expressed in nmol/mg protein.

**Determination of Oxidative Stress Parameters in Kidney**

Kidney tissue was homogenized in approximately 5.0 volumes of ice-cold phosphate buffer using a polytron homogenizer. Homogenates (20% w/v) were then prepared by sonication in ice cold phosphate buffer. Aliquots were prepared and used for the assessment of superoxide dismutase (SOD) and reduced glutathione (GSH) using spectrophotometry by the method of McCord and Fridovich for SOD assessment and by the method of Ellman for GSH assay. Protein content in homogenate was measure by Bradford method.

**Statistical analysis**

Data are presented as mean ± SD and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey or
Bonferroni methods for post-hoc analysis. A value of p<0.05 was considered statistically significant. For statistical analysis, GraphPad 6.01 software was used.

Results

Protective Effect of MGF in DOX-Induced Systemic Toxicity In Vivo

We investigated the effect of MGF on the systemic toxicity caused by DOX through analyzing of weight loss, and the toxicity symptoms such as diarrhea, and spontaneous bleeding. As expected, no physiological alteration was observed, and the increased of BW was detected at the end of the study in the normal group. Conversely, loss of BW occurred in the DOX group. The BW was not different between DOX and DOX+MGF groups, although there was a trend towards of increase in the Co-treatment of DOX and MGF groups. (Figure.1A, B)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mortality Rate (%)</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DOX (n=6)</td>
<td>50</td>
<td>Diarrhea, Bleeding</td>
</tr>
<tr>
<td>DOX+MGF50 (n=6)</td>
<td>28.5</td>
<td>Bleeding</td>
</tr>
<tr>
<td>DOX+MGF100 (n=6)</td>
<td>25</td>
<td>Bleeding</td>
</tr>
</tbody>
</table>

Table 1. Changed of Body weight (BW) and mortality rate.
DOX: Doxorubicin; MGF: mangiferin; Data presented as an average and ±SD (Standard Deviation).

The normal group showed the healthiest appearance compared with other groups, and none of the rats died in this group. However, during the induction of DOX for two weeks, the rats with DOX treatment showed systemic toxicity as revealed by decreasing body weight, diarrhea and spontaneous bleeding in the nose (epistaxis). These phenotypes might be caused by the chronic cytotoxic activity of doxorubicin which causes bone marrow depression, and gastrointestinal malfunction. Therefore, the highest of the mortality rate was detected in rats treated with DOX alone due to diarrhea and spontaneous bleeding (Table. 1). Even the co-treatment with MGF for two weeks has not ameliorated the effects caused by DOX yet but the systemic toxicity was slowly regressed after two weeks of MGF treatment, and the survival rate was improved in both of DOX+MGF50 and DOX+MGF100 groups. (Figure.1C). These data suggest a systemic protective effect of MGF in DOX treatment.

MGF Preserved Kidney Function and Lipid Profile in Rat Treated with DOX

DOX has been known to induce dyslipidemia and renal toxicity represented by elevation of plasma urea and creatinine level, hypoalbuminemia, and proteinuria. Of note, Plasma urea and creatinine levels, and urinary protein excretion were significantly increased in DOX group compared with those in Normal group, whereas those markers were reduced after MGF administration at the dose of 50 and 100 mg/kg BW in DOX+MGF groups (Figure.2A-C). Consistently, previous study reported that accumulative doses of DOX caused kidney dysfunction as shown by increased protein urine excretion and decreased the albumin content in plasma. On the other hand, plasma albumin level was reduced in the DOX group compared with that in Normal group, and MGF treatment significantly improved albumin level in DOX+MGF groups (Figure.2D). Meanwhile, TC and triglyceride levels were significantly decreased in DOX+MGF groups compared with those in DOX groups (Figure.3). These data show that MGF prevent renal failure and dyslipidemia caused by doxorubicin. Similarly, several studies reported that MGF was an effective agent for hyperlipidemia condition and oxidative stress.

Discussion

MGF Inhibited Exacerbation of MDA Induced by Dox

The enhancement of oxidative stress and lipid peroxidation have been known to play a pivotal role in Doxorubicin-induced renal failure which detected by the overproduction of MDA. DOX enters mitochondria and produces reactive oxygen species which cause damage in mitochondrial DNA and later induce respiratory chain dysfunction, which may generate additional reactive oxidative stress that attacks the respiratory chain itself and thus causes further renal lesions. Therefore, we detected that the MDA level in plasma and kidney tissue were increased significantly in DOX-only treated group compared with those in the normal group. During the metabolism process, DOX could generate hydroxyl radical, which causes diffuse to the cell membrane and produce MDA. Therefore, MDA
reacts with DNA and forms the DNA-adduct thus causing DNA damage\textsuperscript{27}. Interestingly, co-treatment of MGF at a dose of 50 and 100 mg and doxorubicin significantly reduced MDA levels in either plasma or kidney tissue (Figure 4A, B) suggesting that MGF inhibit the lipid peroxidation through free radical scavenger action. The ability of MGF as an iron chelating agent that could directly scavenge the free radical also reported in another study\textsuperscript{28}.

**MGF Improved the Antioxidant Activity in Rat Treated with DOX**

We next analyzed the SOD and GSH levels in the kidney to investigate whether MGF enhances the antioxidant activity in rat with doxorubicin treatment. DOX administration caused a significant decreased of SOD and GSH activity levels in kidney tissue compared with that in the Normal group. The mechanism of this phenomenon could be explained by the extensive use of endogenous antioxidant enzymes (SOD and GSH) thus consequently reduce those antioxidant levels. Moreover, treatment with MGF at the dose of 50 and 100 mg/kg BW significantly increased the activity of SOD and GSH levels in kidney tissue compared with those in DOX group (Figure 4C, D) as similar with the other studies which previously reported that MGF improved the antioxidant status in H2O2-induce H9C2 cell and isoproterenol induced myocardial infarction in rat\textsuperscript{28,29}. This improvement might explain by the activity of MGF as a direct free radical scavenger\textsuperscript{25,30}. Together with the previous result, these data collectively suggest MGF protect DOX-mediated systemic and also kidney toxicity through preserving the antioxidant state, particularly in kidney. In the present study, we investigated a protective effect of MGF in the DOX-induced systemic and specific-organ toxicity by inhibition of oxidative stress, particularly in kidney. Consistent with the previous study\textsuperscript{4}, we showed that a cumulative dose of DOX exhibited nephrotoxicity as evidenced by the worsening kidney function such as elevation of serum urea and creatinine, proteinuria, and hypoalbuminemia. Additionally, DOX also induced dyslipidemia represented by increasing plasma TC and triglyceride levels. These pathologies were also associated with excessive production of oxidative stress in both plasma and renal tissues\textsuperscript{4,5}. Moreover, co-
treatment with MGF demonstrated a protective effect towards DOX-mediated nephrotoxicity by increasing antioxidant activities in the kidney thus improve renal function and systemic homeostasis. As our understanding, our study is the first study that reveals the protective effect of MGF on the nephrotoxicity induced by DOX.

**Conclusions**

We found that Co-treatment of DOX and MGF improved renal dysfunction and dyslipidemia in association with the improvement of antioxidant activity level. Thus, our data showed the protective effect of MGF in DOX-induced nephrotoxicity at least in part due to its antioxidant activity.

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**Declaration of Interest**

The Authors declared no conflict of interests.
Figure 1. Body Weight (BW) and Survival Analysis. DOX: Doxorubicin; MGF: Mangiferin; n= 6 each group; Data presented as an average and ± SD (Standard Deviation);*: p: <0.05, **:p:<0.001, ***: p:<0.001, and ****: p: <0.0001. vs. DOX group.
Figure 2. Kidney Function Parameters. DOX: Doxorubicin; MGF: Mangiferin; n= 6 each group; Data presented as an average and ± SD (Standard Deviation);*: p: <0.05, **:p:<0.001, ***: p:<0.001, and ****: p: <0.0001. vs. DOX group.
Mangiferin enhances antioxidant property

Figure 3. Lipid Profiles. DOX: Doxorubicin; MGF: Mangiferin; n= 5-6 each group; Data presented as an average and ± SD (Standard Deviation);*: p: <0.05, **:p:<0.001, ***: p:<0.001, and ****: p: <0.0001 vs. DOX group.

Figure 4. Malondialdehyde (MDA) levels and Antioxidant Enzyme Parameters. DOX: Doxorubicin; MGF: Mangiferin; n= 5-6 each group; Data presented as an average and ± SD (Standard Deviation);*: p: <0.05, **:p:<0.001, ***: p:<0.001, and **:**: p: <0.0001 vs. DOX group.
References


