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ORIGINAL PAPER

Miroestrol, a phytoestrogen from *Pueraria mirifica*, improves the antioxidation state in the livers and uteri of β-naphthoflavone-treated mice

Nattharat Jearapong · Waranya Chatuphonprasert · Kanokwan Jarukamjorn

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Abstract Oxidative stress is involved in the progression of several diseases such as diabetes, hypertension, and age-related diseases. Miroestrol (MR) is a potent phytoestrogen from the tuberous root of *Pueraria mirifica*, a plant used in traditional Thai medicine that is claimed to have rejuvenating effects. In this study, the effects of MR on the antioxidation system, including anti-lipid peroxidation; on the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase; and on glutathione content in the livers and uteri of β-naphthoflavone (BNF)-treated mice were determined. BNF-treated mice are a model of procarcinogen-exposed mice. The results showed that MR improved the antioxidant activities of SOD and CAT in the livers and uteri of both normal and BNF-treated mice, while estradiol (E2) increased SOD activity in the uteri of normal mice and CAT activity in the livers of both normal and BNF-treated mice. In the liver, MR increased the levels of several forms of glutathione, whereas in the uteri E2 and MR reduced the level of lipid peroxidation by decreasing the level of malondialdehyde. Therefore, the use of MR as an alternative hormone replacement therapy might be beneficial due to its ability to improve antioxidation systems.

N. Jearapong · W. Chatuphonprasert · K. Jarukamjorn (✉)
Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Khon Kaen University, Khon Kaen 40002, Thailand
e-mail: kanok_ja@kku.ac.th

N. Jearapong · K. Jarukamjorn
Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

W. Chatuphonprasert
Faculty of Medicines, Mahasarakham University, Mahasarakham 44000, Thailand

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Abbreviations

BNF	β-Naphthoflavone
CAT	Catalase
E2	Estradiol
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
MR	Miroestrol
SOD	Superoxide dismutase

Introduction

Oxidative stress is a harmful process due to the overproduction of free radicals or reactive oxygen species (ROS), which are involved in the aging process and in several types of diseases, including cancer, diabetes, and hypertension [1]. ROS subsequently lead to accumulating damage to the cellular and membrane lipids, proteins, and DNA. Currently, the increase in environmental pollution exposes organisms to ROS from many sources, and ROS are associated with a mechanism of carcinogenesis. Free radicals can be formed from various redox metals and from a well-known carcinogen, tobacco smoke [2]. The endogenous antioxidant system defends cells against these harmful oxygen radicals. There are many enzymes involved in this system, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), in addition to the non-enzymatic glutathione system (GSH/GSSG) [2]. SOD is a major enzyme that catalyzes the

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transformation of ROS into hydrogen peroxide (H_2O_2), which is subsequently detoxified, yielding water (H_2O), by CAT and/or GPx, using GSH as the hydrogen donor. The glutathione system requires glutathione reductase (GR) and $NADP^+$/NADPH to oxidize GSSG to GSH. Glutathione is a major soluble antioxidant in the cytosol, nuclei, and mitochondria of living cells and has a protective role against oxidative stress. The GSH/GSSG ratio has been used as a biomarker of oxidative stress, and a high level of GSSG may indicate damage to many enzymes in the antioxidation system [3]. The protective role of antioxidation enzymes such as SOD, CAT, and GPx against oxidative damage has been found in the context of many diseases. Moreover, free radical production, known as lipid peroxidation, has been shown to be significantly correlated with hepatic carcinogenesis [4].

Recently, phytotherapy has become a popular alternative treatment for diseases, including cancer, and aging. *Pueraria mirifica* (PM) [synonym: *Pueraria candollei* Wall. Ex Benth var. *mirifica* (Airy Shaw & Suvat.) Nyomdham], family Leguminosae, also known as white Kwao-Krua, is a traditional Thai herbal plant that is claimed to have rejuvenating effects, i.e., its use is thought to result in a younger appearance with less skin darkening, re-growth of hair, improved sexual performance, enlargement of the breasts, improved skin smoothness and fewer wrinkles, and a longer lifespan [5]. The potent bioactive compound in the tuberous root of the plant, which exhibits high estrogenic-like activity, is miroestrol (MR) [6]. The effect of MR on the genital tract of female animals has been reported. It was found that a single injection of MR effectively promoted uterine and vaginal growth comparable to that induced by 17β-estradiol and diethylstilbestrol in immature female mice [7]. MR exhibited approximately 0.25 times the estrogenic activity of 17β-estradiol according to a vaginal cornification assay in rats [8]. In a MCF-7 cell proliferation assay, which is based on an estrogen-responsive human breast cancer cell line, MR induced a highly competitive response to an estrogen antagonist [9]. The crude extract of this plant exhibited antioxidant activity in the DPPH assay compared to α-tocopherol [10], and MR was found to have the potential to decrease the level of lipid peroxidation in the mouse brain [6]. Therefore, the potential effect of MR on the activities of antioxidant enzymes is of clear interest because this information has never been presented elsewhere.

The present study aimed to investigate the effect of MR, a potent phytoestrogen isolated from *P. mirifica*, on the activities of antioxidant enzymes, including SOD, GPx, and CAT; the glutathione content; the GSH/GSSG ratio; and the levels of lipid peroxidation in the livers and uteri of β-naphthoflavone (BNF)-treated mice. BNF, a polycyclic aromatic hydrocarbon, is a typical inducer of hepatic cytochrome P450 isoform 1A1, which is involved in carcinogenesis. Therefore, the study of the effect of MR on the anti-oxidation system in BNF-treated mice provided useful information on MR as an estradiol supplement with the ability to protect organs from procarcinogens such as BNF.

Materials and methods

Chemicals

Miroestrol was isolated from tuberous roots of *P. mirifica* as described previously [9, 11]. NMR identification was performed and compared with the authentic standards of MR kindly provided by Dr. Chaiyo Chaichantipyuth, Chulalongkorn University, Thailand. Estradiol benzoate (E2) was purchased from Schering (Kenilworth, NJ, USA). β-Naphthoflavone (BNF), reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH), 4-vinylpyridine (4-VP), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), glutathione reductase, reduced form of glutathione (GSH), oxidized form of glutathione (GSSG), nitrotetrazolium blue chloride (NBT), standard superoxide dismutase (SOD) from bovine erythrocytes, standard malondialdehyde (MDA), standard catalase (CAT), and xanthine oxidase enzyme were supplied by Sigma-Aldrich Chemical (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2) 30 % (w/w) was obtained from Fisher Scientific (Leicestershire, UK). 2-Thiobarbituric acid (TBA) was obtained from Fluka Chemika Co. (Steinheim, Switzerland). All other laboratory chemicals were of the highest purity and from commercial suppliers.

Plant material

The tuberous roots of *P. mirifica* were collected in Ubon Ratchathani, Thailand, in March 2010. Plant materials were identified by Dr. Thaweesak Juengwatanatrakul, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand. Reference specimens (NIPS-KKU 007–010) were deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Animals

Seven-week-old female ICR mice were supplied by the National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand) and housed in the Northeast Laboratory Animal Center, Khon Kaen University, Thailand, under the supervision of a certified laboratory veterinarian. The animal handling and treatment protocols were approved by the Animal Ethics Committee for Use and

Care of Khon Kaen University, Thailand (Approval No. AEKKU 73/2555). At all times, the mice were housed on wood chip bedding in polysulfone cages with water and commercial mouse diet supplied ad libitum and were acclimated for at least 7 days under a 12-h dark/light cycle and controlled temperature (23 ± 2 °C) and humidity (45 ± 2 %) before dosing. Mice (5 per group) were subcutaneously administered daily with E2 at a dose of 0.5 mg/kg/day or MR at doses of 0.5 or 5 mg/kg/day for 7 days. Some mice were given BNF intraperitoneally at a dose of 30 mg/kg/day once a day for the last 3 days. The control group was subcutaneously administered with corn oil (0.1 ml/mouse) once a day for 7 days. All mice were killed 24 h after the last treatment. Livers and uteri were immediately excised and kept at −80 °C for further analysis.

Determination of thiobarbituric acid reactive substances (TBARS) content

Lipid peroxidation was determined by measuring formation of TBARS as described previously [12]. In brief, a 500 µl aliquot of liver or uterus homogenate was incubated at 37 °C for 1 h. Then, 1 ml of an equal volume of 40 % (w/v) trichloroacetic acid (TCA) and 0.2 % (w/v) TBA was added. The reaction mixture was heated at 100 °C for 15 min, before immediately being cooled down. The reaction was then stopped by 40 % (w/v) TCA before centrifuging at 1,123 g at 4 °C for 5 min. The supernatant was measured for TBARS formation by using spectrofluorometry with an excitation wavelength of 528 nm and an emission wavelength of 551 nm compared with a standard of MDA.

Determination of SOD activity

The level of SOD activity was determined based on the degree of inhibition of formation of nitro blue tetrazolium products (formazan) as described previously [11]. The xanthine–xanthine oxidase system was employed as the superoxide generator. In brief, the sample was prepared by adding 120 µl of chloroform and 200 µl of ethanol into 200 µl of the homogenate and then centrifuging the mixture at 13,000×*g* at 4 °C for 30 min. The master mixture, which consisted of 1.12 µM xanthine, 0.14 µM EDTA, 0.14 µM NBT, 55.81 µM Na_2CO_3, and 7.50 µg BSA, was added into the series of standard solutions and samples, and then the xanthine oxidase solution was added. The reaction was incubated at 25 °C for 20 min and then terminated with 0.02 µmol $CuCl_2$ in solution. The absorbance of the formazan product was measured at a wavelength of 550 nm and was used to calculate the percent inhibition by comparison with the blank. The SOD activity was determined by comparison with the percent inhibition caused by the SOD standard.

Determination of CAT activity

The level of CAT activity was determined as described previously [11]. H_2O_2 was employed as the substrate. Briefly, the reaction mixture, which consisted of an aliquot of the sample homogenate and 12.72 µM sodium–potassium phosphate buffer (PBS, pH 7.4), was incubated with 39.39 µM H_2O_2 at 37 °C for 1 min. The reaction was then stopped by the addition of 12.76 mM ammonium molybdate. The absorbance of the yellow product was measured at a wavelength at 405 nm. The CAT activity (kU/L) was calculated based on the percent inhibition of the molybdate–H_2O_2 complex using a standard curve constructed with hepatic bovine CAT.

Determination of total glutathione content, ratio of GSH to GSSG, and GPx activity

The total glutathione (GSH) content was analyzed as previously described [11]. 4-VP was employed to determine the GSSG content. The homogenates were deproteinized with 5 % (w/v) 5-sulfosalicylic acid (SSA) and then kept at 2–8 °C for 10 min before centrifuging at 10,000*g* at 4 °C for 10 min. To determine the total GSH content, the supernatant was mixed with a freshly prepared reaction mixture containing 95 mM potassium phosphate buffer (pH 7.0), 0.95 mM EDTA, 0.04 mg/ml NADPH, 0.04 mg/ml DTNB, 0.12 units/ml glutathione reductase, and 0.24 % SSA. The reaction was started by adding NADPH, and the absorbance at a wavelength of 405 nm (A_{405}) was immediately measured every 60 s for 5 min. The total GSH content was determined by comparing the A_{405}/min (slope) of the sample with the series of the slope of the GSH standard. The reduced GSH content was calculated by subtracting the GSSG content from the total GSH content. To determine the GSSG content, 4-VP was added to the supernatant, and the sample was incubated for 60 min at room temperature before performing the assay [11].

The level of GPx activity was determined as described previously [13] with some modifications [11]. The reaction mixture, which consisted of the sample homogenate, 2.48 mM sodium phosphate buffer (pH 7.4), 0.02 mM EDTA, and 8.26 µM sodium azide, was incubated at 30 °C for 10 min, followed by the addition of 1.24 mM GSH. The reaction was started by adding 1.24 mM H_2O_2 and then stopped by adding 3.31 % (w/v) SSA. The reaction mixture was centrifuged at 330 g for 15 min. The supernatant was employed for the determination of GPx activity as described for GSSG content. The value was expressed in mUnit/mg protein, and a unit of GPx was defined as the

amount of GPx that produces 1 μmol of GSSG/min at 30 °C and pH 7.4.

Statistical analysis

The results were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (version 11.5; SPSS Inc., Chicago, IL, USA). $p \leq 0.05$ was considered to be statistically significant.

Results

Effect of miroestrol on TBARS level in mouse livers and uteri

To determine whether MR has an antioxidative effect on oxidative stress, the level of lipid peroxidation was determined in mouse livers and uteri based on the MDA content produced in the TBARS reaction. BNF did not change the level of MDA in the livers, whereas the MDA levels were significantly increased by both E2 and MR (Fig. 1a). The co-administration of MR with BNF dose-dependently elevated the level of MDA in the livers. Likewise, an increase in the hepatic MDA level was produced by co-treatment with E2 and BNF. In contrast to the MDA levels in the livers, the level of MDA was significant decreased in the uteri by all single treatments (BNF, E2, and MR), and the effect of MR was dose-dependent (Fig. 1b). In addition, both E2 and MR significantly reduced the MDA level in the uteri of BNF-treated mice. These results indicate that E2 and MR prevent oxidative stress in mouse uteri.

Effect of miroestrol on SOD activity in mouse livers and uteri

The antioxidant effect of MR on SOD activity in the livers and uteri of female mice was examined based on the inhibition of formazan formation. Neither BNF nor E2 altered the level of SOD activity in the livers, whereas the SOD activity was greatly increased by MR in a dose-dependent manner (Fig. 2a). The co-administration of BNF with E2 significantly reduced the level of SOD activity, whereas the SOD activity was greatly elevated by co-treatment with BNF and MR at a dose of 5 mg/kg/day. In the uteri, even though treatment with BNF or E2 alone enhanced the level of SOD activity, co-treatment with BNF and E2 resulted in no improvement in the SOD activity (Fig. 2b). MR did not increase the level of SOD activity. Interestingly, the co-administration of BNF and MR significantly increased the SOD activity.

Effect of miroestrol on CAT activity in mouse livers and uteri

The antioxidant activity of the CAT enzyme was investigated based on the formation of the yellow complex of molybdate and H_2O_2. The level of hepatic CAT activity was not affected by BNF (Fig. 3a), whereas it was significant raised by treatment with E2 or MR in a dose-

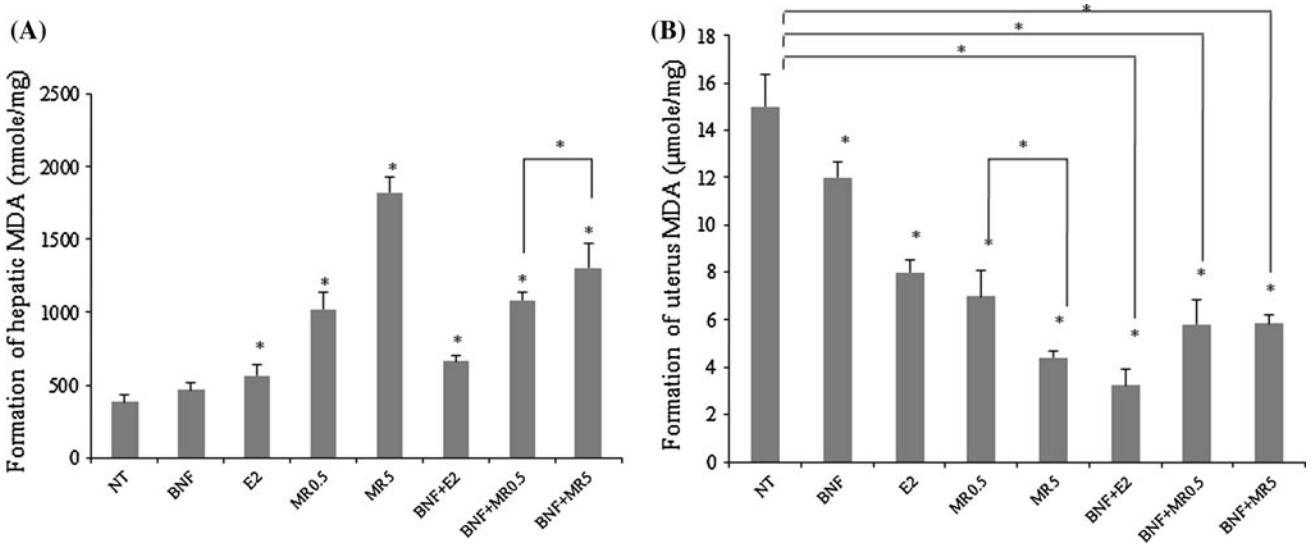

Fig. 1 Effect of miroestrol on lipid peroxidation in mouse livers (**a**) and uteri (**b**). Female ICR mice were administered daily with estradiol (*E2*, 0.5 mg/kg/day), or miroestrol (*MR*, 0.5 or 5 mg/kg/day) for 7 days, and/or β-naphthoflavone (*BNF*, 30 mg/kg/day) for the last 3 days. The data are presented as mean ± SD ($n = 5$). Significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. * $p < 0.05$

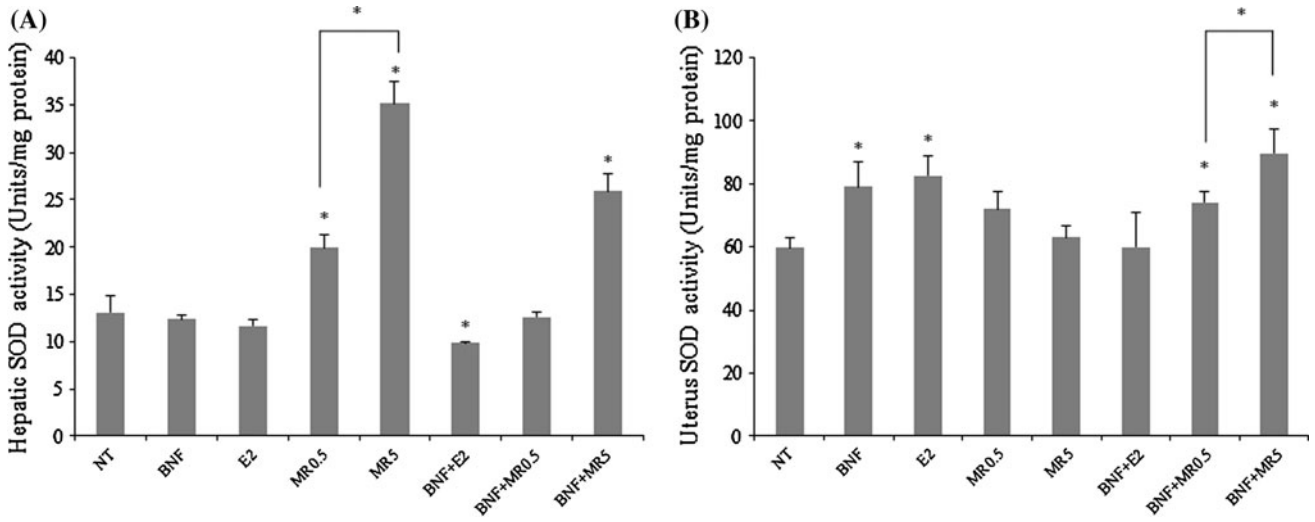

Fig. 2 Effect of miroestrol on levels of SOD activity in mouse livers (**a**) and uteri (**b**). Female ICR mice were administered daily with estradiol (*E2*, 0.5 mg/kg/day), or miroestrol (*MR*, 0.5 or 5 mg/kg/day) for 7 days, and/or β-naphthoflavone (*BNF*, 30 mg/kg/day) for the last 3 days. The data are presented as mean ± SD ($n = 5$). Significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. * $p < 0.05$

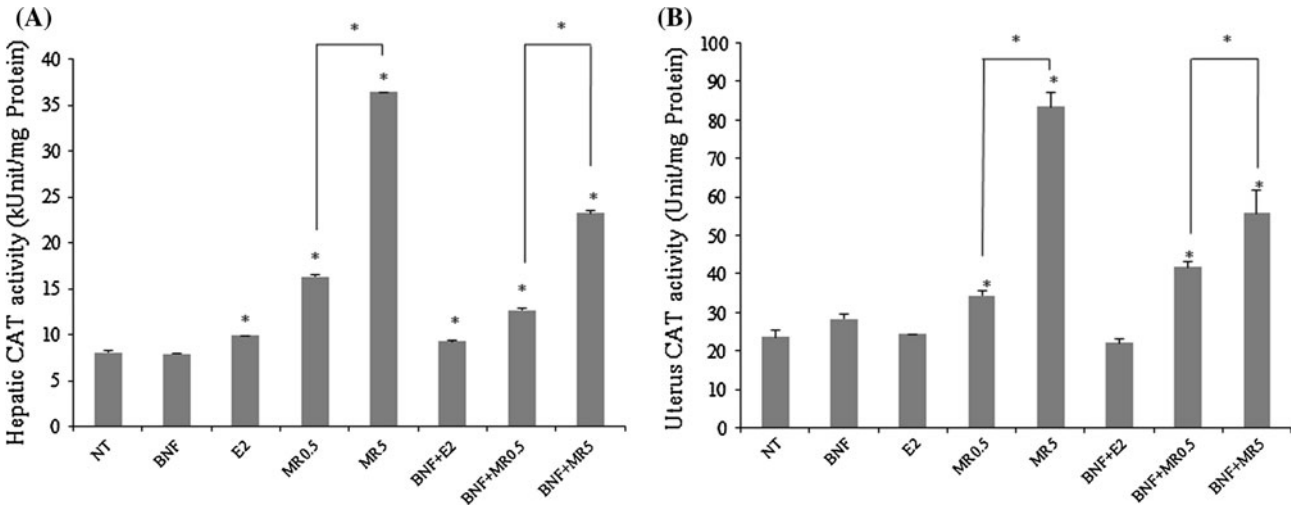

Fig. 3 Effect of miroestrol on levels of CAT activity in mouse livers (**a**) and uteri (**b**). Female ICR mice were administered daily with estradiol (*E2*, 0.5 mg/kg/day), or miroestrol (*MR*, 0.5 or 5 mg/kg/day) for 7 days, and/or β-naphthoflavone (*BNF*, 30 mg/kg/day) for the last 3 days. The data are presented as mean ± SD ($n = 5$). Significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. * $p < 0.05$

dependent manner. The enhancing effect of the high dose of MR (5 mg/kg/day) was markedly higher that the effect of E2. The co-administration of either MR or E2 with BNF also significantly elevated the hepatic CAT activity. In the uteri, neither BNF nor E2 resulted in a significant increase in the CAT activity, whereas this activity was notably increased by MR (Fig. 3b). The co-administration of BNF and E2 did not change the CAT enzyme activity relative to the level in the untreated group. Treatment with BNF combined with MR improved the CAT activity, although the increase was not as high as that induced by treatment with MR alone.

Effect of miroestrol on GPx activity, total glutathione content, and the ratio of GSH to GSSG in mouse livers

The level of GPx activity; the contents of total glutathione, reduced glutathione (GSH), and oxidized glutathione (GSSG); and the GSH/GSSG ratio were determined based on the amount of yellow product generated from the reaction of GSH with DTNB. BNF increased the total glutathione content by increasing both the GSH and GSSG levels in the livers (Table 1), but there were no significant changes in the GSH/GSSG ratio or the GPx activity. E2 did not affect either the GSH or GSSG level, although the total

Table 1 Effect of miroestrol on levels of total glutathione (total GSH), reduced glutathione (GSH), and oxidized glutathione (GSSG) contents, and the ratio of reduced/oxidized glutathione (GSH/GSSG), and glutathione peroxidase (GPx) activity in mouse livers

Treatment group	Total GSH (nmol/mg)	GSH (nmol/mg)	GSSG (nmol/mg)	Ratio of GSH/GSSG	GPx (mUnit/mg/min)
Livers					
NT	16.43 ± 0.95	10.56 ± 0.95	5.87 ± 0.09	1.80 ± 0.15	36.13 ± 4.97
BNF (30 mg/kg/day)	23.43 ± 0.76*	15.76 ± 0.76*	7.67 ± 0.01*	2.06 ± 0.10	47.74 ± 11.48
E2 (0.5 mg/kg/day)	19.04 ± 0.42*	12.79 ± 0.42	6.25 ± 0.57	2.05 ± 0.08	26.59 ± 6.54
MR0.5 (0.5 mg/kg/day)	52.60 ± 2.02*	36.15 ± 2.02*	16.57 ± 0.10*	2.18 ± 0.13*	47.34 ± 13.33
MR5 (5 mg/kg/day)	31.49 ± 0.26*	21.96 ± 0.26*	9.53 ± 0.00*	2.30 ± 0.07*	42.44 ± 15.26
BNF + E2	14.39 ± 0.29	9.40 ± 0.29	4.99 ± 0.24*	1.90 ± 0.20	10.93 ± 3.41*
BNF + MR0.5	31.67 ± 0.27*	22.08 ± 0.27*	9.58 ± 0.22*	2.30 ± 0.03*	20.09 ± 11.06
BNF + MR5	47.60 ± 0.85*	32.16 ± 0.85*	15.44 ± 0.23*	2.08 ± 0.08	61.87 ± 16.19*

The data are presented as mean ± SD ($n = 5$)

NT non-treatment, *BNF* β-naphthoflavone, *E2* estradiol benzoate, *MR* miroestrol

* $p < 0.05$ vs. NT

glutathione content was increased. In addition, E2 did not alter the GSH/GSSG ratio or the GPx activity relative to the levels in the control livers. MR significantly increased the levels of all glutathione fractions, including total glutathione, GSH, and GSSG, and increased the GSH/GSSG ratio without changing the GPx activity. Significant reductions in the GSSG content and the GPx activity were observed after the co-administration of BNF and E2, but the GSH/GSSG ratio was not affected. The combination of BNF and MR significantly increased both the GSH and GSSG contents in a dose-dependent manner and resulted in an increase in the GSH/GSSG ratio. In addition, the GPx activity was significantly elevated by co-treatment with BNF and a high dose of MR (5 mg/kg/day). Regarding the glutathione contents in the uteri, only the total glutathione content of some groups could be determined (data not shown).

Discussion

Oxidative stress involves the excessive production of free radicals, leading to damage to lipids, proteins, and nucleic acids. Antioxidants function as defense mechanisms against oxidative injury by scavenging superoxide from endogenous and exogenous sources, yielding harmless products [14]. In the present study, we investigated the protective role of MR against oxidative stress, including lipid peroxidation, and the ability of MR to enhance the antioxidation system. We assessed both the enzyme system, i.e., CAT, SOD, and GPx, and the non-enzyme system, i.e., the glutathione system, in the livers and uteri of BNF-treated female ICR mice.

In post-menopausal women, aged-related oxidative stress plays a key role in menopause-related symptoms, in the decrease in physiological functions, and in the depletion of estrogen [15]. These symptoms and pathological processes include hot flashes, arteriosclerosis, skin aging, and a decrease in bone density [16]. Therefore, the beneficial effects of MR on the antioxidant protection system suggest that it could be used as an alternative hormone replacement therapy for postmenopausal women by attenuating oxidative injury associated with the pathological processes.

The biochemical defense mechanisms against free radicals involve the glutathione system and the primary enzymatic antioxidants SOD, CAT, and GPx [14]. Under normal conditions, these enzymes act co-operatively, and there is a balance between both the activities and levels of these antioxidants in cells [2]. This balance is essential; if there is any change in either the activities or levels of these enzymes, oxidative stress may occur. SOD is the first line of defense in the antioxidation system against oxidative stress and catalyzes the transformation of the highly reactive superoxide anion into H_2O_2 [14]. An increase in the antioxidation activity indicates a higher capacity to scavenge H_2O_2 in tissues and to decrease superoxide content in cells [2]. Our observations show that MR significantly increased the activities of SOD and CAT in mouse livers, although in the uteri only the CAT activity was raised. E2 elevated the CAT activity in the livers and the SOD activity in the uteri. This phenomenon might be explained via the several factors regulating uterus SOD activity, such as the age of the animal, the state of the menstrual cycle, and the antioxidant property of a compound. The study of Moorthy et al. [17] revealed that the effects of hormone replacement therapy on the uterine SOD activity were dependent on the ages of the rats and the period of treatment (varied from 12 to 24 months). In the present study, the mice were given MR for only 7 days, and hence the treatment period might not be enough to observe the changes. The activity of SOD was possibly different in

human uteri due to the phases of endometrium cycles [18]. In addition, the study of Kinalski et al. [19] noted the potency of vitamin E as an antioxidant in diabetic rats, in that it improved SOD activity in the rat livers, but not in the rat uteri. Therefore, it is not required that an antioxidant compound influences all antioxidation enzymes in the organs investigated in the same pattern or to the same degree. GPx is an enzyme that metabolizes H_2O_2 into H_2O using GSH as a hydrogen or electron donor [14]. A reduction in the level of GPx activity results in the accumulation of toxic products and leads to oxidative damage [2]. The GSH/GSSG ratio indicates the level of oxidative damage in human disease states [3]. The GSH system is a non-enzymatic system that protects against the toxic effects of lipid peroxidation [2, 14]. In the present study, MR enhanced the protective non-enzymatic system by increasing the GSH/GSSG ratio in the livers, although elevated GPx activity was not noted. The generation of free radicals results in oxidative stress and damage to cell phospholipids. Lipid peroxidation is a harmful reaction involving free radicals, and the final product is called MDA [20]. Consistent with the results of previous studies [6, 21], both E2 and MR exhibited dose-dependent effects on lipid peroxidation in mouse uteri, with a greater effect for MR. E2 and MR increased the formation of MDA in the livers, whereas BNF did not. These effects might be explained by the fact that BNF is a flavonoid derivative that has antioxidation properties due to its structure. In addition, E2 and MR are exogenous compounds, and the liver is the primary detoxification organ. The formation of MDA occurs after treatment with E2 or MR. These results indicate that E2 and MR can prevent oxidative stress in mouse uteri, but the hepatotoxicity should be noted, especially at high doses of MR.

Our very recent report demonstrated that MR downregulated the expression of the *Cyp1a2* gene at both the mRNA and enzymatic levels [6]. CYP1A is the major cytochrome P450 subfamily of hepatic enzymes that participates in the metabolism of potential procarcinogens, such as polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, and aryl and heterocyclic arylamines, into their active forms [22]. CYP1A1 is constitutively expressed in several extrahepatic tissues, whereas CYP1A2 is expressed only in the liver. BNF, a typical CYP1A inducer through aryl hydrocarbon receptor activation [23], was employed to simulate the activation of a procarcinogen to evaluate the antioxidant effects of MR. The hepatic SOD and GPx activities in BNF-treated mice were significantly elevated by MR at the dose of 5 mg/kg/day. These findings strongly suggest that MR improves the antioxidation status of the livers and uteri of BNF-treated mice.

The reduction in the level of lipid peroxidation in the mouse uteri by BNF is not consistent with the results of the study by Chatuphonprasert et al. [12], in which a significant increase in the level of MDA was observed in the livers of male C57BL/6 mice. This difference might be explained by the different mouse strains used; C57BL/6 is an Ah-responsive inbred mouse strain [24], whereas ICR is an outbred mouse strain. These mouse strains might have different degrees of sensitivity to BNF. In addition, the effect of BNF might be suppressed due to estrogenic activity in females [23]. Moreover, the decrease in MDA formation after a short period of BNF exposure may result from the chemical structure of BNF, which is a flavonoid derivative [25]. Although MR had a protective effect against lipid peroxidation in the uteri, an increase in the hepatic MDA level was noted for both treatment with E2 or MR alone and co-administration of BNF and either E2 or MR. These findings might be the result of the fact that the liver is the major metabolizing organ; therefore, the liver is the primary organ exposed to xenobiotics and has a higher risk of organ damage. Thus, the use of the optimal dose of MR is critical, and the titration of the optimal dose of MR is required.

Conclusion

The beneficial effects of MR on the enzymatic system in both mouse livers and uteri result in lower levels of free radicals, which may be involved in carcinogenesis and aging processes. MR at the optimal dose possessed antioxidation effects, protecting against lipid peroxidation and enhancing the activities of antioxidation enzymes in the livers and uteri of female mice. These findings support the use of MR as an alternative estrogen replacement therapy instead of estradiol in post-menopausal women.

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Conflict of interest The authors have no conflict of interest.

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