

Bromelain has Antioxidant Effect On Methotrexate Hepatotoxicity and Nephrotoxicity

Bromelain Metotreksat Hepatotoksisitesi ve Nefrotoksisitesi Üzerine Antioksidan Etkilidir

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ÖZET

Amaç: Bu çalışmada bromelainin (BRM) metotreksat (MTX) kaynaklı böbrek ve karaciğer hasarına karşı potansiyel koruyucu etkilerinin araştırılması amaçlandı.

Materyal ve Metot: Dört gruba ayrılan sıçanlarda (n=7); kontrol grubuna 14 gün gavaj yoluyla distile su ve üçüncü gün intraperitoneal (i.p.) fizyolojik salin (%0.9 NaCl) verildi; BRM grubuna 14 gün boyunca gavaj yoluyla 200 mg/kg BRM ve üçüncü gün i.p fizyolojik salin (%0.9 NaCl); MTX grubuna 14 gün boyunca gavaj yoluyla distile su ve üçüncü günde i.p. tek doz 20 mg/kg MTX; BRM+MTX grubuna 14 gün boyunca gavaj yoluyla 200 mg/kg BRM ve üçüncü günde i.p. tek doz 20 mg/kg MTX uygulandı. Deney sonunda sıçanlar dekapite edildi, böbrek ve karaciğer dokuları -80 °C'de muhafaza edilerek, doku homojentlerinden elde edilen supernatantlarda biyokimyasal analizler gerçekleştirildi.

Bulgular: MTX uygulaması ile oksidasyon belirteci tiyobarbitürik asit reaktif madde (TBARS) seviyeleri kontrol grubuna kıyasla arttı; antioksidanlar süperoksit dismutaz (SOD), glutatyon (GSH), glutatyon peroksidaz (GPx) ve katalaz (CAT) aktiviteleri kontrol grubuna göre azaldı. BRM'nin MTX ile birlikte uygulanması, TBARS seviyesinde azalmaya ve GSH, CAT, SOD ve GPx aktivitelerinde artışa neden oldu.

Sonuç: Bu çalışmada MTX'in böbrek ve karaciğer dokularında oksidatif hasara neden olduğu ve BRM'nin bu hasarı önlediği belirlenmiştir.

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Anahtar Kelimeler: Metotreksat, Oksidatif hasar, Böbrek, Karaciğer, Bromelain.

ABSTRACT

Objective: The goal of this study was to look into whether bromelain (BRM) could protect against methotrexate (MTX)-induced kidney and liver damage.

Material and Method: The rats were divided into four groups (n=7); the control group was given distilled water by gavage for 14 days and intraperitoneal (i.p.) physiological saline (%0.9 NaCl) on the third day; the BRM group was given 200 mg/kg BRM by gavage for 14 days and i.p. physiological saline (%0.9 NaCl) on third day; the MTX group was given distilled water by gavage for 14 days and i.p. single dose of 20 mg/kg MTX on the third day; the MTX+BRM group was given 200 mg/kg BRM by gavage for 14 days and i.p. single dose of 20 mg/kg MTX on the third day. Rats were decapitated at the end of the experiment, kidney and liver tissues were kept at -80°C, and biochemical analyzes were performed on the supernatants obtained from tissue homogenates.

Results: With the administration of MTX, oxidation indicator thiobarbituric acid reactive substance (TBARS) levels increased in comparison with the control group; antioxidants- glutathione peroxidase (GPx), glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities were decreased compared to the control group. Co-administration of BRM with MTX resulted in reduction in TBARS level and increase in GSH, CAT, SOD, and GPx activities.

Conclusion: In this study, it was determined that MTX caused oxidative damage in kidney and liver tissues and BRM prevented this damage.

Keywords: Methotrexate, Oxidative damage, Kidney, Liver, Bromelain.

INTRODUCTION

Methotrexate (MTX) is a folic acid antagonist that works by inhibiting the dihydrofolate reductase enzyme. It's used to treat cancer and autoimmune inflammatory disorders like rheumatoid arthritis, as

well as gynecological pathologies like ectopic pregnancy and gestational trophoblastic diseases (Hafez et al., 2015). Serious side effects observed in various organs and tissues, including the gastrointestinal tract, liver, kidneys, and nervous system limit the

use of MTX. Oxidative stress and inflammatory processes have been linked to these adverse effects (Bernsen et al., 2020). Interference of MTX with folate metabolism, deterioration of intracellular metabolic pathways and mitochondrial enzymes by polyglutamated forms of MTX mediate these adverse effects (LaCasce, 2021).

The pineapple from the Bromeliaceae family grows in the equatorial region; it is a plant with anti-cancer, anti-inflammatory, antioxidant, and anti-platelet effects (El Demerdash et al., 2020). Bromelain (BRM) is obtained from pineapple; it is a mixture of carbohydrates and various thiol endopeptidases, glucosidases, phosphatases, cellulases, peroxidases, glycoproteins and protease inhibitors, however the mode of its action is not properly understood (Pavan et al., 2012). BRM, which is absorbed from the intestines without losing its biological effectiveness, has been reported to have positive effects in various diseases such as surgical traumas, platelet aggregation inhibition, thrombophlebitis, sinusitis, pyelonephritis, bronchitis, and angina pectoris. (White et al., 1988; Castell et al., 1997; Pavan et al., 2012).

The goal of this study was to see if BRM could protect kidney and liver cells from oxidative damage caused by MTX. We assessed the antioxidant markers glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) activity, as well as the oxidative stress marker thiobarbituric acid reactive substance (TBARS).

MATERIAL and METHOD

Chemicals: Methotrexate (Koçak Farma, 500 mg/20 ml) and BRM (Solgar, 500 mg, Leonia, New Jersey) were purchased from the pharmacy and all other chemicals were of analytical grade or the highest purity available and were obtained from Sigma-Aldrich.

Experimental animals and study design: Ethics committee approval of the study was received from Adiyaman University Animal Experiments Ethics Committee (31.10.2019- 2019/050). 28 Sprague Dawley male rats were used. During the study, feed and

water were given ad libitum to the rats, which were housed in polypropylene cages with a 12-hour light-dark cycle and a constant temperature of 21°C.

Twenty eight rats were divided equally into four groups as 7 rats in each group. The rats in the control group were given distilled water by gavage once a day for 14 days and injected a single dose of physiological saline i.p. on the third day. The rats in the BRM group were given 200 mg/kg BRM by gavage once a day for 14 days, and a single dose of physiological saline was injected i.p. on the third day. The rats in the MTX group were administered distilled water by gavage once a day for 14 days with distilled water and on the third day a single dose of 20 mg/kg MTX was administered i.p. The rats in the MTX+BRM group were administered 200 mg/kg BRM once a day for 14 days by gavage and on the third day a single dose of 20 mg/kg MTX by i.p. injection. At the end of the study, rats that were administered general anesthesia with a xylazine-ketamine mixture were decapitated. Kidney and liver tissues were rapidly removed and stored at -80 °C for biochemical measurements.

Biochemical measurements: Tissues were homogenized in 0.2 M Tris-HCl buffer (pH: 7.4) under cold chain conditions to achieve a 1:10 (w/v) dilution of the entire homogenate. The direct homogenate was used for TBARS measurements. SOD, CAT, and GPx activities and GSH levels were determined from the supernatants obtained by centrifuging the homogenates at 3220 rpm for 30 min (4 °C). TBARS levels, a lipid peroxidation index, were measured using Yagi's method (Yagi, 1998). The samples were evaluated spectrophotometrically by measuring at 532 nm and the results were shown as nmol/g tissue. SOD, CAT, and GPx are members of the cellular enzymatic antioxidant defense system. SOD serves as the first line of defense against free oxygen radicals by converting superoxide anion to hydrogen peroxide, and decreasing CAT and GPx hydrogen peroxide to water in mitochondria. (Ighodaro and Akinloye, 2018). The spectrophotometric method was used to determine SOD, CAT, and GPx activities and results were

expressed as units/mg tissue protein. For determining SOD activity, method of Sun et al. (Sun et al., 1998) was used. In this method, which is based on the inhibition of nitroblue tetrazolium (NBT) reduction caused by the superoxide radical produced by the xanthine/xanthineoxidase system, the enzyme activity that inhibits NBT reduction by 50% is accepted as 1 SOD activity. CAT activity was determined by the method of Aebi (Aebi, 1974) based on the principle of monitoring the degradation of hydrogen peroxide in the environment by the effect of CAT as a decrease in absorbance at 240nm wavelength. The absorbance difference per unit time was used as a measure of CAT activity. GPx activity was determined according to the method of Paglia and Valentine (Paglia and Valentine, 1967). In this method, GPx, which reduces hydrogen peroxide to water, converts the reduced glutathione into its oxidized form. In the presence of glutathione reductase and NADPH, oxidized glutathione is reduced back to reduced glutathione. The decrease in NADPH in the medium is followed as a decrease in absorbance at 340 nm. The decrease in absorbance is proportional to the activity of GPx. GSH is one of the members of the second line antioxidant defense system. It scavenges free radicals by donating them electrons. GSH levels were determined spectrophotometrically by measuring at 412 nm wavelength according to the method of Sedlak and Lindsay (Sedlak and Lindsay, 1968). Tissue GSH levels were expressed as nmol/mg tissue protein.

The Lowry method was used to determine the amount of tissue protein (Lowry et al., 1951).

Statistical Analysis: Statistical analyzes were performed using the IBM SPSS 21 program. Whether the data showed normal distribution or not was analyzed with the Shapiro-Wilk test. One-way analysis of variance (ANOVA) test was used to compare variables between groups. According to Levene test analysis, Tukey HSD was used for variables with homogeneous variance and Games-Howell test was used for variables with non-homogeneous variance as post hoc tests. The mean and standard error of the results were calculated (SEM). Statistical significance was defined as $p < 0.05$ value (95 percent confidence interval).

RESULTS

Kidney and liver tissue TBARS and GSH levels and SOD, GPx and CAT activities are given in tables 1 and 2. In our study, administration of MTX increased TBARS levels in kidney tissue compared to the control group; and caused decrease in SOD GPx, GSH and CAT activities without statistical significance. Co-administration of BRM with MTX caused a significant reduction in TBARS level compared to MTX group; and caused increase in SOD, GPx, GSH and CAT activities although without statistical significance.

Table 1. Changes in TBARS, SOD, GPx, GSH and CAT levels in kidney tissues of rats treated with MTX and BRM

Kidney	TBARS (nmol/g)	GSH (nmol/mg)	SOD (U/mg)	GPx (U/mg)	CAT (U/mg)
Control	88,68±0,37 ^a	18,00±1,37	3,95±0,28	0,91±0,06	444,44±20,15
BRM	73,95±1,20 ^b	20,03±1,61	4,16±0,54	1,03±0,08	450,58±11,28
MTX	92,28±0,65 ^a	17,09±1,16	3,96±0,13	0,90±0,04	414,22±6,77
MTX+BRM	82,98±0,95 ^c	17,60±1,09	4,33±0,42	0,96±0,05	439,69±19,94

Means bearing different superscripts within same column were significantly different ($P < 0,05$). Mean±SEM.

In comparison with the control and BRM groups, MTX administration resulted in a large increase in TBARS levels in liver tissue, as well as a significant decrease in GSH, SOD, GPx, and CAT activities. In comparison to the MTX group, administration of

BRM with MTX resulted in a large decrease in TBARS levels, as well as a considerable increase in GSH, SOD, GPx, and CAT activities as shown in the Table 2.

Table 2. Changes in TBARS, SOD, GPx, GSH and CAT levels in liver tissues of rats treated with MTX and BRM.

Liver	TBARS (nmol/g)	GSH (nmol/mg)	SOD (U/mg)	GPx (U/mg)	CAT (U/mg)
Control	22.21±1.75 ^a	8.51±0.58 ^a	2.66±0.14	0.84±0.04 ^a	1490.99±61.27 ^a
BRM	23.18±2.40 ^a	8.24±0.75 ^a	2.75±0.22	0.87±0.05 ^a	1456.67±81.52 ^a
MTX	43.15±4.29 ^b	4.30±0.31 ^b	2.32±0.06	0.57±0.03 ^b	1076.87±53.74 ^b
MTX+BRM	31.53±0.73 ^a	7.54±0.21 ^a	2.45±0.09	0.75±0.01 ^a	1252.18±30.62 ^{ab}

Means bearing different superscripts within same column were significantly different ($P < 0.05$). Mean±SE.

DISCUSSION

MTX, a folate antimetabolite cytotoxic antineoplastic agent, is used in the treatment of many malignancies. It is also used in the treatment of rheumatoid arthritis and some other rheumatic and inflammatory diseases due to its immunosuppressive/modulatory effects (Wang et al., 2018). MTX-polyglutamates formed after entering the cell potently inhibit the dihydrofolate reductase enzyme. Inhibition of the dihydrofolate reductase enzyme stops protein synthesis by blocking the synthesis of thymidines and purines, which need tetrahydrofolate for their synthesis (Wang et al., 2018). Methotrexate causes serious side effects in many tissues and organs, especially in tissues with high proliferation, that limit its use. Hepatotoxicity, nephrotoxicity, pulmototoxicity, hematotoxicity, neurotoxicity, cardiotoxicity, gastrointestinal, gonadal toxicity, and neocarcinogenic effects are the main side effects (Campbell et al., 2016). Side effects depend on dose, administration route, and frequency of administration and concomitant use of folic acid.

Kidneys and liver are organs responsible for homeostasis, toxin/metabolite/drug elimination and com-

plex metabolic functions in the organism. Methotrexate-induced nephrotoxicity and hepatotoxicity are attributed to the pathological processes of oxidative damage, inflammation, and apoptosis (Yalçın and Gürel, 2020).

In this study, administration of 20 mg/kg single dose of MTX caused an increase in TBARS levels and a decrease in SOD, GPx, GSH, CAT activities in rat kidney and liver tissues. These results were consistent with the literature detecting oxidative damage caused by MTX (Yalçın and Gürel, 2020).

BRM is a mixture of sulfur-containing enzymes isolated from pineapple. BRM, which is absorbed without losing its biological activity and deteriorating after oral administration and has no side effects even with long-term use (Agarwal et al., 2016). Furthermore, BRM's antioxidant properties have been established in earlier research. Oral administration of 250 mg/kg BRM against aluminum-induced oxidative damage significantly decreased TBARS and H₂O₂ levels and increased GSH, SOD, CAT and GPx activities in rat kidney tissue (El Demerdash et al., 2020) and in rat testis tissue (Jebur et al., 2020).

Agarwal et al. (Agarwal et al., 2016) showed that administration of 70 mg/kg BRM to mice exposed to

dichlorvos toxicity significantly reduced serum TBARS and protein carbonyl content- another indicator of oxidative stress, and increased GSH level and SOD, CAT, and GPx activities.

In our study, 200 mg/kg oral BRM administration increased GSH, GPx, CAT, and SOD activities compared to the MTX administered group. As a result, the application of BRM provided partial improvement against MTX-induced oxidative kidney and liver damage. Therefore, BRM may be useful in preventing nephrotoxicity and hepatotoxicity that may occur in people receiving MTX therapy.

Conflict of Interest: No conflict of interest declared by the authors.

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