RESEARCH ARTICLE

Hepatoprotective effect of *Zataria Multiflora* Boiss against malathion-induced oxidative stress in male rats

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Abstract This study was aimed to evaluate the protective effects of methanolic extract of *Zataria Multiflora* Boiss (*Z. multiflora*) against oxidative stress induced by malathion (MT) in male Wistar rats in comparison to N-acetylcysteine (NAC). Rats were randomly divided into 6 groups (n: 8) and treated daily for 28 days. They received MT (150 mg/kg), *Z. multiflora* methanolic extract (50, 100, and 200 mg/kg), and NAC (200 mg/kg) alone or in combination. This study included a histopathological examination of liver tissue in parallel with measurement of serum aminotransferases. Activities of antioxidant enzymes, total glutathione (GSH), and lipid peroxidation end products in plasma and tissue were also measured. Subacute exposure of rats to MT resulted in a significant morphological change in tissue, an increase in plasma aminotransferases, and oxidative damage. *Both NAC and Z. multiflora at the dose of 200 mg/kg significantly reduced MT-induced oxidative stress by inhibiting lipid peroxidation, increasing the content of glutathion, and restoring the activities of superoxide dismutase and catalase.* Biochemical and histological observations also showed a dose dependent hepatoprotective effect of *Z. multiflora* extract against subacute exposure to MT.

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Introduction

Environmental pollution contributes to pathogenesis of many diseases affecting plants, animals, and humans. Today, organophosphorus (OPS) compounds have become a dangerous environmental pollution because of various agricultural and household irrational uses. Primary effect of OPS in mammals is inhibition of acetylcholinesterase (AChE) at nerve synaptic junctions and accumulation of acetylcholine. However, OPS can lead to a wide range of toxicity including induction of oxidative stress, mitochondrial damages, and metabolic disorders (Karami-Mohajeri and Abdollahi 2011; Karami-Mohajeri and Abdollahi 2013).

Malathion (MT) as an OPS compound can cause physiological, biochemical, immunological, and histological changes in vivo and in vitro (Karmakar et al. 2016). Besides AChE inhibition, induction of oxidative stress is another mechanism of toxicity that may be due to redox-cycling activity of MT and generation of reactive oxygen species (ROS). In recent years, many studies have suggested that antioxidants such as N-acetylcysteine (NAC), Vitamin E, selenium, and herbal antioxidants can decrease oxidative damages caused by MT (Aboul-Soud et al. 2011; Fortunato et al. 2006; Moore et al. 2010; Mostafalou et al. 2012; Selmi et al. 2015).

Zataria Multiflora Boiss (*Z. multiflora*), belonging to the family Labiatae, is native to Iran and is used traditionally in food as a stimulant, condiment, and carminative (Sharififar et al. 2007). Medicinal properties of this plant such as antioxidant (Dashipour et al. 2015; Sharififar et al. 2011), antimicrobial, analgesic (Hosseinzadeh et al. 2000) and anti-inflammatory properties have also been reported (Nakhai

et al. 2007). Antioxidant effects of *Z. multiflora* are contributed to flavonoids and polyphenolic compounds found in its methanolic extract and act as reducing agents. Thymol and carvacrol are non-polar phenolic compounds in this herb for pharmacological effects (Sharififar et al. 2007). The antioxidant capacity of these compounds are attributed to the fact that they are cofactors of antioxidant enzymes and electron donors (Sharififar et al. 2011).

The aim of the present study was to find out protective effects of methanolic extract of *Z. multiflora* against oxidative stress induced by MT in male Wistar rats in comparison to NAC as a well-known antioxidant.

Materials and methods

Chemicals

For this study, technical MT 95 % was obtained from Shimi-Keshavarz company. Other chemical with high purity were purchased from Sigma-Aldrich company.

Animals

Adult male Wistar rats weighing 250 ± 10 g were kept on a 12-h-light/12-h-dark cycle and at room temperature with free access to tap water and standard laboratory chow diet. All animal experiments were approved by the Ethical Committee of the Kerman Neuroscience Research Center (EC/KNRC/92–30) that was completely in agreement with the "Guide for Care and Use of Laboratory Animals, DHEW Publication Done (NIH) 85-23, 1985" (Ethical code: IR.KMU.REC.1394.310).

Collection and preparation of plant extracts

Z. multiflora plants were collected from the mountain of Shiraz city and authenticated by professor Fariba Sharififar. Amount of 5000 g fresh aerial parts of the plants were washed, dried at room temperature, and powdered. Sequential extraction of leaf was carried out using petroleum ether, chloroform, and methanol. Finally, the methanolic extract were evaporated at 45 °C with a rotary evaporator and then the resulted dry powder of extract (712 g) were collected and stored in a refrigerator at 4 °C for further use.

Animal treatment

Animals were randomly divided into 6 groups of 8 animals and treated for 28 days. Rats of negative and positive control groups received, respectively, corn oil (0.1 mg/kg) and MT (150 mg/kg). Animals of experimental groups received MT (150 mg/kg) in combination with *Z. multiflora* extract (50, 100, and 200 mg/kg) or NAC (200 mg/kg) by gavage.

Twenty four hours after the last dose, all animals were anesthetized with ketamine and xylazine and 2 ml of blood were taken through cardiac puncture. Serum and plasma were separated and rapidly frozen at −70 °C for later biochemical analysis. After blood sampling, all animals were killed and liver tissues were taken for oxidative damage and histopathological examination.

Histopathological analysis

Liver tissue were fixed in 10 % formalin solution and then processed for embedding in paraffin using conventional methods. Sections (5 μm) were stained with hematoxylin and eosin and observed under a microscope for evaluating of histopathological changes and taking photomicrographs.

Serum aminotransferases levels

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were estimated according to 2,4-dinitrophenylhydrazine method described by Reitman and Frankel. The absorbance was measured at 546 nm by spectrophotometery (Reitman and Frankel 1957).

Determination of lipid peroxidation

Lipid peroxidation was estimated using a method based on the measurement of malondialdehyde (MDA) as a major product of lipid peroxidation was estimated (Garcia et al. 2005). MDA was determined by thiobarbituric acid (TBA) reaction method. Supernatant of liver homogenate was mixed with 2 volumes of TBA reagent (15 % trichloroacetic acid, 0.8 % TBA, 0.25 N HCl) and heated at 95 °C for 15-min to form MDA-TBA adduct. Optical density was measured with a spectrophotometer from BioTek™ instruments, Inc. (Winooski, Vermont, USA) at 532 nm. MDA was calculated using molar extinction coefficient of MDA as 1.56×10^5 and expressed as nmole/g of tissue protein.

Determination of total thiols

Total thiols were quantified spectrophotometrically using Ellman's method little modification (Jollow et al. 1974). 0.25 ml of solution (10 mM 5,5-dithionitrobenzoic acid in 0.1 M phosphate buffer, pH 8) were added to 0.5 ml of sample (at least 2 nmol of protein). After incubation in the dark for 15-min at room temperature, the absorbance was measured at 412 nm. Total thiol content was calculated using glutathione (GSH) standard curves and expressed as mmol/g of tissue protein.

Measurement of catalase (CAT) activity

The activity of CAT was measured as described previously by Johansson and Borg (Johansson and Borg 1988). Briefly, 1 ml of 30 mM H_2O_2 and 50 µl of the sample was added to 2 ml of 50 mM phosphate buffer (pH 7.0) and then the absorbance was measured kinetically at 240 nm. The concentration of H_2O_2 was calculated using the following expression: H_2O_2 (mM) = (Absorbance 240 nm × 1000)/molar extinction coefficient (43.6 M^{-1} cm^{-1}). Finally, the specific activity was expressed as U/g of tissue protein, where 1 unit is the amount of CAT necessary to decompose of 1 µM of H_2O_2 in 1-min under standard conditions.

Measurement of superoxide dismutase (SOD) activity

Pyrogallol autoxidation method as described previously by Marklund and Marklund (Marklund and Marklund 1974) was used for measurement of SOD activity. The autoxidation rate of 2 mM pyrogallol in Tris-HCl buffer (pH 8.2) was determined kinetically at 420 nm alone and after addition of 50 µl of sample. Amount of SOD needed for 50 % inhibition of the pyrogallol autoxidation was considered as one unit of SOD activity and expressed as U/g of protein tissue.

Statistical analysis

Data were analyzed by using commercially available SPSS Software. Data analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Results were presented as means ± SEM (Standard error of mean) and *p*-values less than 0.05 were regarded as statistically significant.

Results

Histopathological findings

Light microscopic observation of control group showed normal morphology of liver tissue with well-designed hepatocytes and sinusoids. The euchromatin nucleus of hepatic cells had a normal vesicular structure and cytoplasm appeared uniform and normal (Fig. 1a).

In the group treated with MT, gross degeneration and severe necrotic lesions were observed in periportal lobules (Fig. 1b), along with cytoplasmic vacuolation around nuclei (Fig. 1c). Lesion in centrilobular area was less severe, but sinusoid expansion and atrophy of hepatocytes were found (Fig. 1d).

Severity of lesions in the group which received methanolic extract of *Z. multiflora* (50 and 100 mg/kg) with MT was much reduced, and hepatocytes showed a hydropic degeneration in periportal area (Fig. 1e and f). Notably, these histopathological changes were significantly decreased in the group which received *Z. multiflora* (200 mg/kg) and NAC (100 mg/kg) (Fig. 1g and h).

Serum aminotransferase levels

Serum activities of hepatic enzymes, AST and ALT, significantly increased in MT-treated rats after 28 days, but returned to normal values by treatment with *NAC and Z. multiflora* methanolic extract at the oral dose of 200 mg/kg. However, there was no significant difference in serum AST and ALT after treatment with 50, 100 mg/kg *(*Table 1*)*.

Oxidative stress biomarkers

Subacute exposure to MT increased concentration of MDA in liver tissue which was significantly reduced by *NAC and Z. multiflora* at the dose of 200 mg/kg. Effectiveness of different doses of *Z. multiflora* extract on lipid peroxidation induced in liver by MT is shown in Fig. 2.

As shown in Fig. 3, GSH content decreased in livers of rats treated with MT and was significantly restored to normal level by NAC and *Z. multiflora* (200 mg/kg). *Z. multiflora* at the doses of 50 and 100 mg/kg showed a trend towards elevating the GSH content but did not reach statistical significance.

Results of examinations on the activities of antioxidant enzymes, CAT and SOD, are presented in Figs. 4 and 5. Results showed the sublethal dose of MT decreased the activities of these enzymes in relation to the control group. *NAC and Z. multiflora* at the dose of 200 mg/kg significantly returned the activities of antioxidant enzymes back to their control levels. But the dose of 50 and 100 mg/kg resulted only in small changes in activities of CAT and SOD.

Discussion

MT is a widely used pesticide that affects various organs especially liver. Studies have shown that MT significantly increase the levels of AST, ALT, Alkaline phosphatase (ALP), and LDH, which reflects its hepatotoxicity potential in rats (Abdollahi et al. 2004; Rahimi and Abdollahi 2007). In this study, a significant increase in AST and ALT was also observed after administration of MT. In addition, MT induced severe hepatic damages as shown in histopathological examination which coupled with markedly increased levels of hepatic biomarkers (ALT, AST, and ALP) in blood. On the other hand, administration of *Z. multiflora* significantly decreased both histopathological changes in liver tissue and serum aminotransferases activities.

The amount of MDA as the products of cell membrane lipid peroxidation were also increased in liver tissue of rats received MT. Lipid peroxidation is involved in the
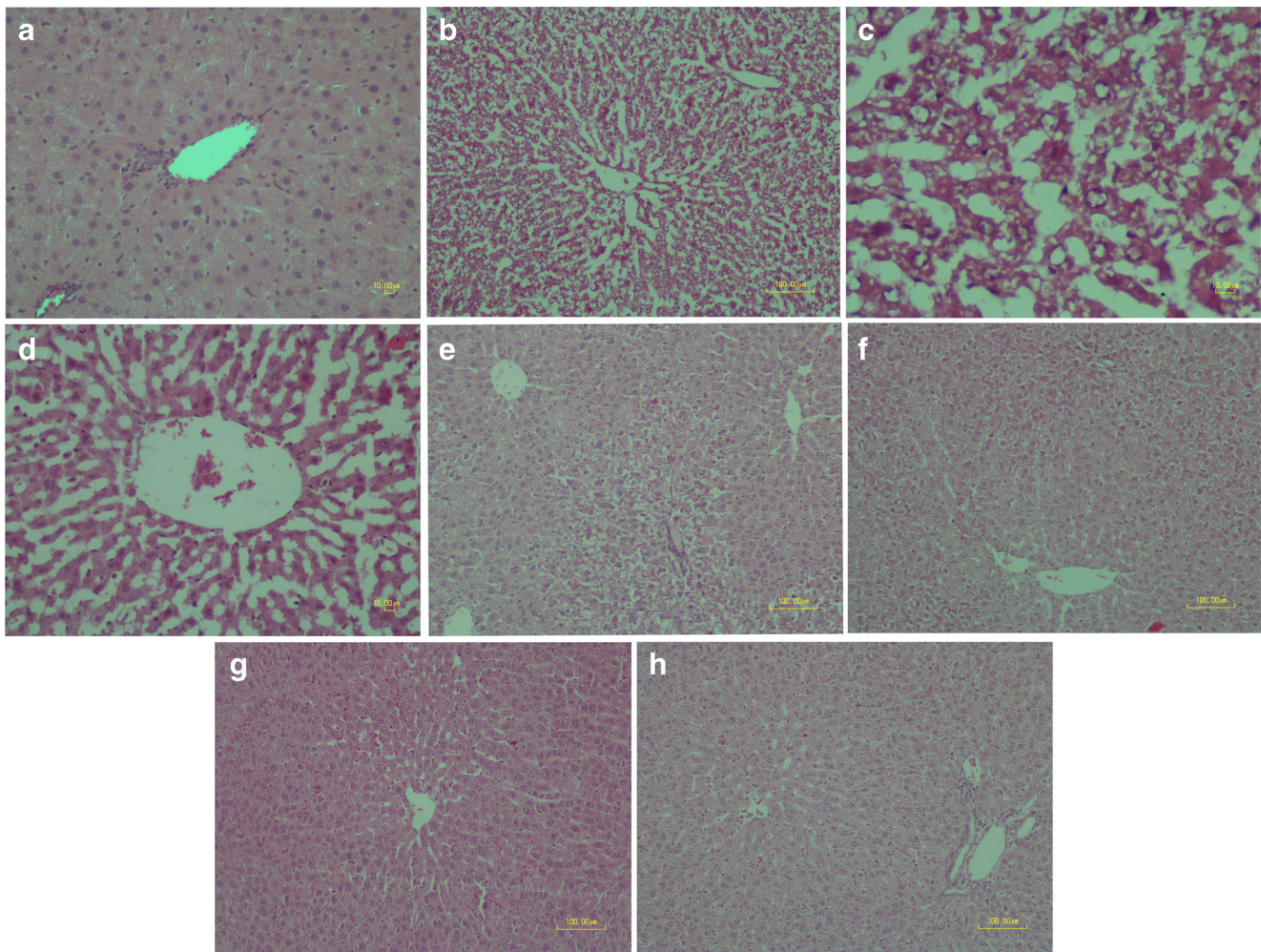
Fig. 1 Photomicrographs of liver sections obtained from 1**a** Normal group, 1**b**,1**c**,1**d** Malathion (150 mg/kg) group, 1**e** Malathion (150 mg/kg) + *Z. multiflora* (50 mg/kg) group, 1**f** Malathion (150 mg/kg) + *Z. multiflora* (100 mg/kg), 1**g** Malathion (150 mg/kg) + *Z. multiflora* (200 mg/kg) group, and 1**h** Malathion (150 mg/kg) + NAC (100 mg/kg) group

pathogenesis of liver damage caused by MT by decreasing cell membranes integrity and release of the hepatic enzymes into blood stream (Abdollahi et al. 2004). Treatment by *Z. multiflora* prevented lipid peroxidation which can be attributed to presence of antioxidant ingredients in methanolic extract of this plant and its radical scavenging activity (Nakhai et al. 2007; Samarghandian et al. 2016; Sharififar et al. 2011; Sharififar et al. 2007).

Nonenzymatic antioxidant GSH redox homeostasis is a major intracellular ubiquitous regulator in all cell types

(Hayes et al. 2005; Lomaestro and Malone 1995). In current study, MT administration led to a significant decline in the content of GSH in liver tissue, which can be a compensatory response to reduce toxicity of MT. Lee and et al. show that GSH plays an important role in removing toxic metabolites leading to liver histopathological injuries (Lee et al. 2008). Treatment with multiple doses of *Z. multiflora* increased levels of GSH, probably due to the increase of GSH synthesis enzymes such as the synthesis of *gamma*-glutamylcysteine and glutathione S-transferase, which needs further study.

Table 1 Effect of various doses of *Z. multiflora (ZM) (50, 100, and 200 mg/kg) and* N-acetylcysteine *(*NAC) (200 mg/kg) on the activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in comparison to control group (Ctrl) and group received malathion (MT)

	Ctrl	MT	MT + ZM 50	MT + ZM 100	MT + ZM 200	MT + NAC
ALT	119.0 ± 10.76	190.5 ± 8.42***	177.3 ± 6.78***	161.2 ± 9.30*	118.3 ± 5.85	111.3 ± 6.83
AST	25.50 ± 3.70	81.17 ± 9.45***	70.50 ± 6.47***	60.17 ± 7.70**	38.50 ± 4.67	29.33 ± 6.28

Data expressed as Mean $\pm$ SEM; $n = 8$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, were compared with the control group (one-way ANOVA followed by Tukey's multiple comparison test)
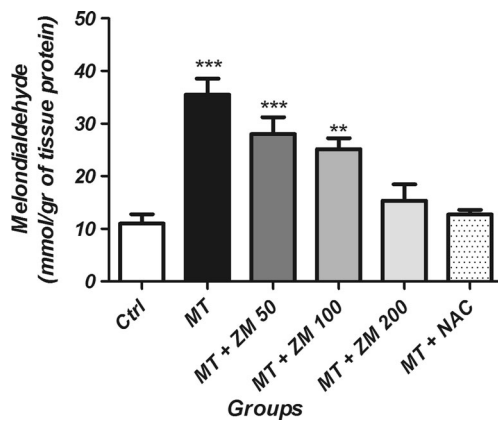
Fig. 2 Effect of various doses of *Z. multiflora* on the lipid peroxidation of liver tissue in comparison to positive and negative control groups. Data are shown as mean ± SEM and the number of rats in each group was 6. Results with $p < 0.05$ was considered significant. **$*P < 0.01$; ***$P < 0.001$

Fig. 4 Effect of various doses of *Z. multiflora* on the activity of catalase enzyme in comparison to positive and negative control groups. Data are shown as mean ± SEM and the number of rats in each group was 6. Results with $p < 0.05$ was considered significant. *$P < 0.05$; **$P < 0.01$; ****$P < 0.001$

Hepatoprotective effect of *Z. multiflora* methanolic extract against the toxicity of MT may be because of restoring of GSH in liver tissue.

Many studies have been focused to evaluate the activities of the antioxidant enzymes such as SOD and CAT as the endogenous enzymatic defense system. These enzymes in combination with nonenzymatic antioxidants convert the ROS to harmless metabolites. SOD accelerates dismutation of superoxide anion to H_2O_2 and O_2. As H_2O_2 is still harmful to cells, CAT further accelerates decomposition of H_2O_2 to water (Hayes et al. 2005). MT administration decreased the antioxidant capacity and decreased the activity of antioxidant enzymes in the livers of rats which were in agreement with previous reports (Fortunato et al. 2006; Possamai et al. 2007). Treatment with *Z. multiflora* significantly increased the activity of antioxidant enzymes in comparison to the group received MT only. Nakhai and et al. have shown the positive effect of different dose of *Z. multiflora* in the activities of
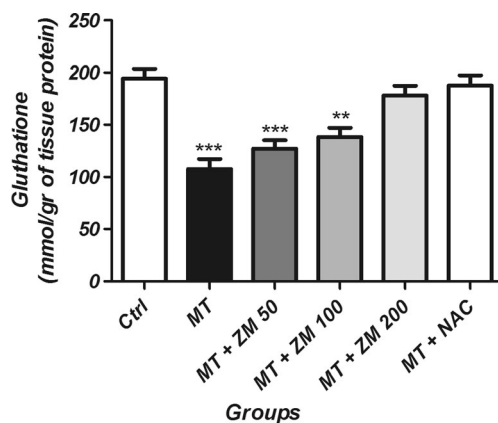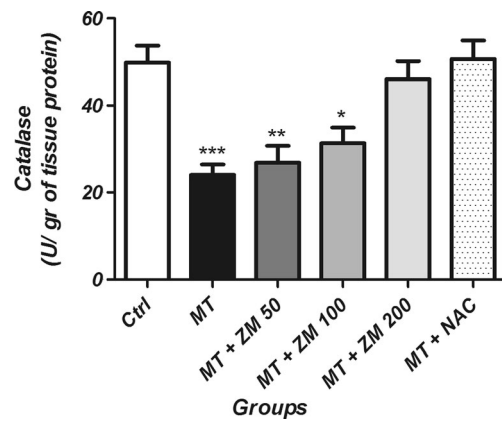
myeloperoxidase as an antioxidant enzymes in experimental model of mouse inflammatory bowel disease by reduction of oxidative damages (Nakhai et al. 2007).

We can concluded that despite the different mechanisms of MT toxicity, the main cause of hepatotoxicity of MT is induction of oxidative stress. The antioxidant compounds such as NAC in the treatment of poisoning caused by pesticides, especially organophosphates, are important. Therefore, identification and purification of these antioxidants will be helpful in planning for future therapeutic strategies. Hepatoprotective effects of *Z. multiflora* may be the result of its free radical scavenging activity as well as NAC. *Z. multiflora* especially at the dose of 200 mg/kg increase the antioxidant capacity of the liver, due to its bioactive antioxidants and its capacity in improving the enzymatic antioxidants defense system. Previous studies also show hepatoprotective effect of *Z. multiflora* methanolic extract against oxidative damage occurred in different diseases (Fatemi et al. 2012; Hosseinzadeh
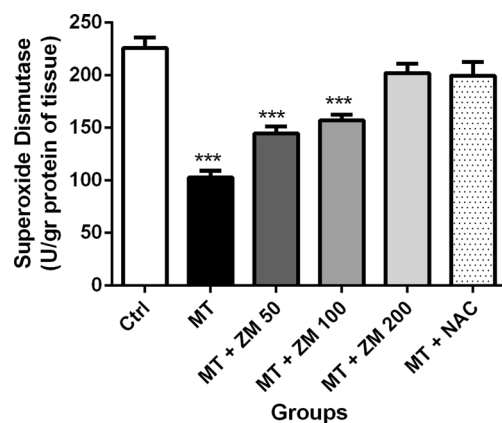

Fig. 3 Effect of various doses of *Z. multiflora* on the glutathione content of liver tissue in comparison to positive and negative control groups. Data are shown as mean ± SEM and the number of rats in each group was 6. Results with $p < 0.05$ was considered significant. **$P < 0.01$; ***$P < 0.001$

Fig. 5 Effect of various doses of *Z. multiflora* on the activity of superoxide dismutase enzyme in comparison to positive and negative control groups. Data are shown as mean ± SEM and the number of rats in each group was 6. Results with $p < 0.05$ was considered significant. ***$P < 0.001$

et al. 2000; Nakhai et al. 2007; Sajed et al. 2013). It has been confirmed that plants nonenzymatic antioxidant compounds such as phenols, flavonoids, and tannins are free radicals scavengers and these compounds can delay or inhibit oxidative damages (Gill and Tuteja 2010; Madrigal-Santillán et al. 2014; Rajesh et al. 2015; Ruberto and Baratta 2000). So, further studies are needed to find out, extract and purify compounds with the antioxidant activity in *Z. multiflora* methanolic extract.

Compliance with ethical standards

Conflict of Interest The authors declare there is no conflict of interest.

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