Archives of Razi Institute, Vol. 77, No. 3 (2022) 1083-1089

DOI: 10.22092/ARI.2022.357266.2005

Original Article

Effect of *Moringa oleifera* Leaves against Hepatotoxicity Induced by Bisphenol A

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Received 27 December 2021; Accepted 16 January 2022
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Abstract

Bisphenol A (BPA) is a synthetic compound with alterations in the liver, antioxidant enzymes, and reproductive hormones. The therapeutic potential of *Moringa oleifera* extract has recently been considered. The present study aimed to estimate the leaf extract of *M. oleifera* against hepatotoxicity induced by BPA. In total, 44 adult male rats were used in this study, and the experiment was conducted on 11 groups (4 animals per group). The rats were administrated (orally) with 5 and 10 mg/kg BPA and treated (orally) with 100, 200, 300, and 400 mg/kg of the aqueous extract of *M. oleifera.* After 28 days of challenge, liver enzymes, including aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as a pathological study using the liver tissue sections were determined. The findings showed a significant ($P \leq 0.05$) increase in the AST, ALT, and ALP in the BPA groups with different histological changes that included the sclerosis of the bile duct surrounded by fibrocytes and lymphocytes infiltration. After treatment with *M. oleifera*, the liver enzymes and tissue returned to a normal state and showed non-significant ($P \leq 0.05$) differences, compared to the control group. According to the results, it can be concluded that the aqueous extract of *M. oleifera* has a great potential to prevent and improve liver damage of BPA.

Keywords: Bisphenol A, Liver enzymes, *Moringa oleifera*

1. Introduction

The compound called Bisphenol A (BPA) is defined as a synthetic high production monomer utilized in polycarbonate plastics and the resins of epoxy in consumer products. These products are used in canned beverages and food, bottles made of plastic, plastic food containers, thermal receipts, plastic medical equipment, and various plastic consumer products used in other applications (1-7). Regarding the health effect, BPA leads to hepatotoxic effects, mutagenic features for DNA, carcinogenic effects, and direct effects on the reproductive system (8-10). Moreover, Lee, Kwon (11) have indicated that BPA causes DNA strand breaks in the lymphoma cells of the mouse. In addition, BPA has the ability to induce various liver lesions and kidney tissue changes with brain injuries and other organs with histopathological changes by generating reactive oxygen species (ROS) (12). *Moringa oleifera* (belongs to family *Moringaceae*) is a fast-growing evergreen with the leaves form as tripinnate, possesses fragile branches, generally grows to reach 10-12 m in length (13, 14), and is distributed in African and Asian countries (15). The leaf powder of *M. oleifera* is rich in various minerals, such as iron, vitamin A, and vitamin C, which are significant to iron metabolism (16, 17). *M. oleifera* is indicated to be utilized in the treatment of different diseases, including rheumatism and ascites, as well as viral diseases (e.g., influenza virus), bacterial

infections, and different types of abscess (18). Many studies indicated disease prevention by using the leaves of *M. oleifera*, which have the ability to reduce hyperglycemia effect and dyslipidemia (19). Furthermore, the leaf extract prevented DNA damage in mice (20) and promoted hepatic glutathione restoration (21). Therefore, this study aimed to estimate the leaf extract of *M. oleifera* against hepatotoxicity induced by BPA.

2. Materials and Methods

2.1. Preparation of the Aqueous Extract of *M. oleifera*

M. oleifera leaves powder was stored at 4°C, and 15 gm powder was extracted using 350 ml of distilled water. It was then filtered via Whatman paper. The mixture was evaporated to obtain aqueous extract and then kept in glass at -20°C until utilization (22).

2.2. Experimental Design

In total, 44 adult male rats (weight: 150-180 mg; age: 3-7 months) were obtained from the Veterinary College, University of Tikrit, Tikrit, Iraq.

The rats were divided into 11 groups (4 animals per group) after the challenge with BPA and evaluated with different concentrations (100, 200, 300, and 400 mg/kg) of the leaf extract as follows. BPA was prepared from Alpha Chemica, India, and used orally in rats. Two different concentrations of 5 mg and 10 mg were used to create toxic effects of BPA. The aqueous extract of *M. oleifera* was administered orally at different concentrations either before, with, or after treatment with BPA once a day for 28 days.

A. Rats were administrated (orally) with 1 ml of normal saline (the control group).

B. Rats were administrated (orally) with 5 mg/kg BPA (Alpha Chemical, India).

C. Rats were administrated (orally) with 10 mg/kg BPA.

D. Rats were administrated (orally) with 5 mg BPA and treated with 100 mg/kg.

E. Rats were administrated (orally) with 5 mg BPA and treated with 200 mg/kg.

F. Rats were administrated (orally) with 5 mg BPA and treated with 300 mg/kg.

G. Rats were administrated (orally) with 5 mg BPA and treated with 400 mg/kg.

H. Rats were administrated (orally) with 10 mg BPA and treated with 100 mg/kg.

I. Rats were administrated (orally) with 10 mg BPA and treated with 200 mg/kg.

J. Rats were administrated (orally) with 10 mg BPA and treated with 300 mg/kg.

K. Rats were administrated (orally) with 10 mg BPA and treated with 400 mg/kg.

2.3. Liver Enzymes Assay

After the challenge, 5 ml blood samples were taken from the rats, and the isolated serum was used to analyze liver enzymes. Aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined by using the reagent kits (Fuji film device with AST, ALT ALP-kits, France).

2.4. Histological Study

The rat liver samples were used for pathology analysis. The liver was fixed in 10% formalin until the preparation for the histological section. The liver slides were prepared and stained by Hematoxylin and Eosin (23).

2.5. Statistical Analysis

The statistical analysis was conducted using the one-way ANOVA, followed by the least significant difference test. In addition, the data of the study were expressed as mean±SD, and the statistical significance was set at $P \leq 0.05$ (24).

3. Results

3.1. Liver Enzymes

The AST levels showed significant ($P \leq 0.05$) differences in the groups of BPA (5 mg and 10 mg), compared to the control group. After treatment with *M. oleifera*, AST levels returned to a normal range and showed non-significant ($P \leq 0.05$) differences, compared to the control group as shown in figure 1. Furthermore, the ALT levels showed significant ($P \leq 0.05$) differences in the groups of BPA (5 mg and 10 mg), compared to the control group. After treatment with *M. oleifera*, the

ALT levels returned to a normal range and showed non-significant ($P \leq 0.05$) differences, compared to the control group as shown in figure 2. In addition, the ALP levels showed significant ($P \leq 0.05$) differences in the groups of BPA (5 mg and 10 mg), compared to the control group. After treatment with *M. oleifera*, the ALP levels returned to a normal range and showed non-significant ($P \leq 0.05$) differences, compared to the control group as shown in figure 3.

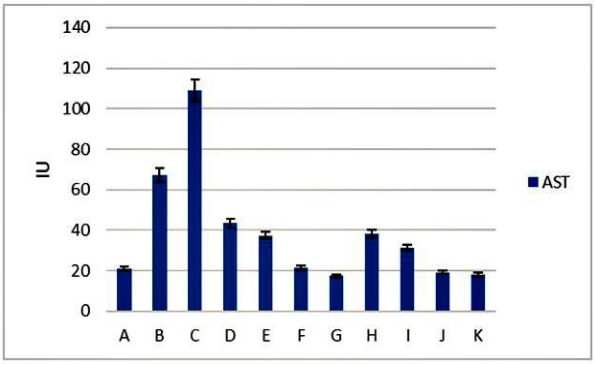

Figure 1. Levels of AST in the studied groups

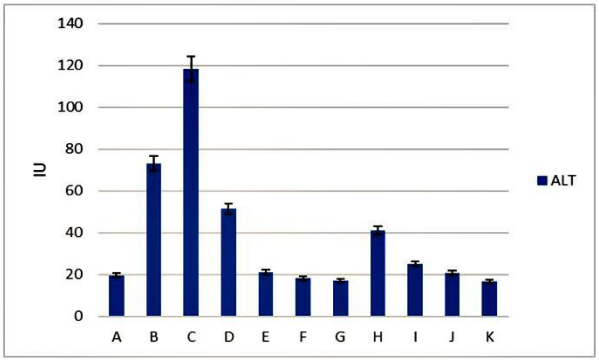

Figure 2. Levels of ALT in the studied groups

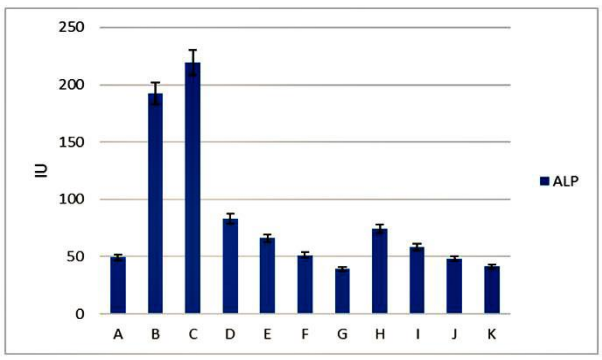

Figure 3. Levels of ALP in the studied groups

3.2. Histological analysis

Regarding the control group, figure 4 illustrates the normal structure of the central vein, radial arrangement of hepatocytes around the central vein, the normal diameter of sinusoids, and Kupffer cells. The BPA group (5 mg) showed the thickening wall of the central vein with congestion and sclerosis of the bile duct that was surrounded by fibrocytes and lymphocytes infiltration with hemolysis (Figure 5). After treatment, the group of 5 mg BPA with 100 mg *M. oleifera* showed the thickening wall of the central vein with congestion and sclerosis of the bile duct that was surrounded by fibrocytes and lymphocytes infiltration (Figure 6). However, BPA treated with 200 mg (Figure 7), 300 mg (Figure 8), and 400 mg (Figure 9) of *M. oleifera* showed that the liver returned to the normal tissue. Moreover, the liver sections of these groups showed normal central vein, hepatocytes, sinusoids, and Kupffer cells. Figure 10 of the BPA group (10 mg) shows the thickening wall of the central vein with congestion and sclerosis of the bile duct that is surrounded by fibrocytes and lymphocytes infiltration with hemolysis. After treatment, the group of 10 mg BPA with 100 mg (Figure 11) and 200 mg (Figure 12) of *M. oleifera* showed the thickening wall of the central vein with congestion. However, the BPA treated with 300 mg (Figure 13) and 400 mg (Figure 14) showed that the liver returned to normal tissue. Moreover, the liver sections of these groups showed normal central vein, hepatocytes, sinusoids, and Kupffer cells.

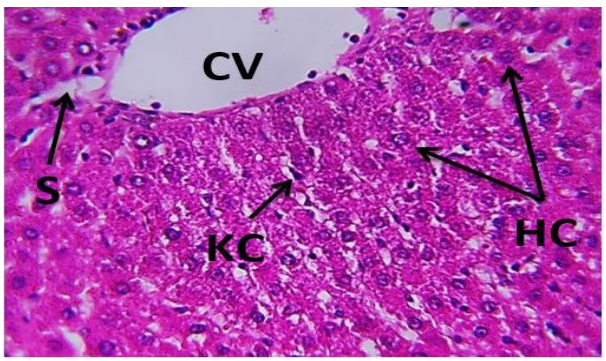

Figure 4. Liver of control group show normal structure of central vein (CV), hepatocytes (HC), sinusoids (S) and Kupffer cells (KC) H&E X400

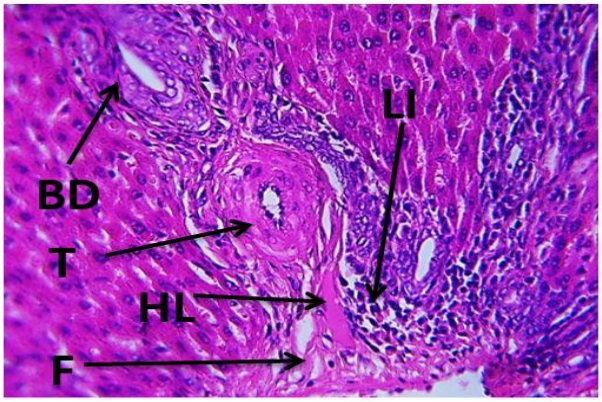

Figure 5. Liver of 5mg Bis group show thickening wall (TW) of central vein with congestion (CON), sclerosis of bile duct (BD) that surrounded by fibrocytes (F) and lymphocytes infiltration (LI) with hemolysis (HL) H&E X400

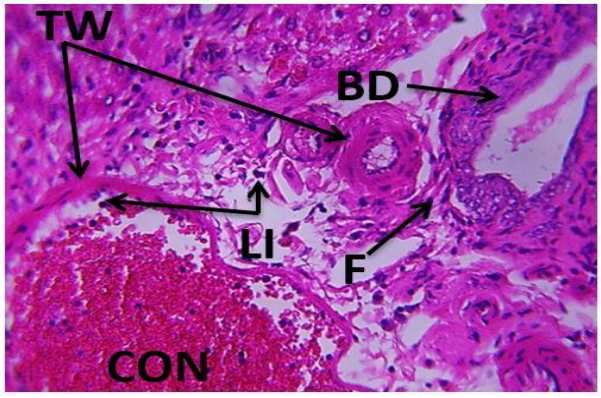

Figure 6. Liver of 5mg Bis & 100mg MOLP group show thickening wall (TW) of central vein (CV) with congestion (CON), sclerosis of bile duct (BD) that surrounded by fibrocytes (F) and lymphocytes infiltration (LI) H&E X400

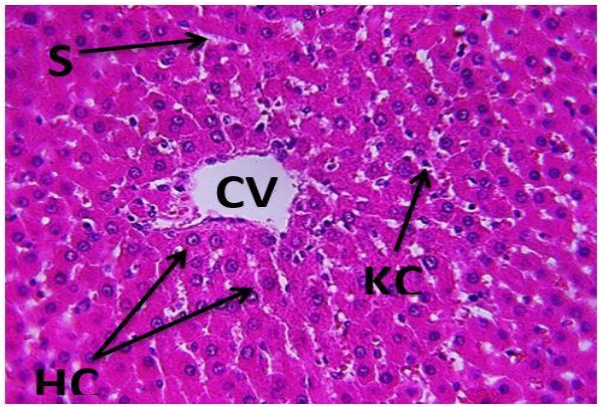

Figure 7. Liver of 5 mg Bisphenol and 200 mg MOLP group show central vein (CV), hepatocytes (HC), sinusoids (S), and Kupffer cells (KC) (H&E ×400)

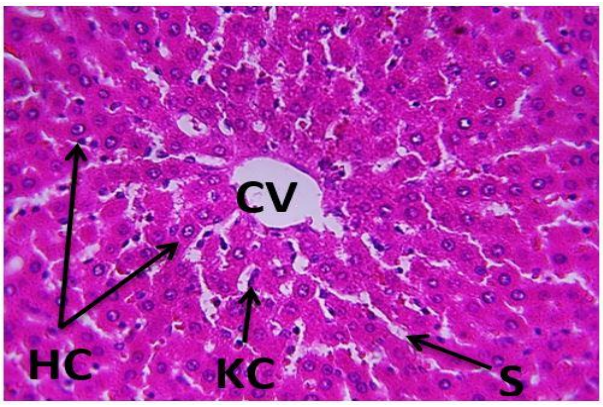

Figure 8. Liver of 5mg Bis & 300mg MOLP group show central vein (CV), hepatocytes (HC), sinusoids (S) and Kupffer cells (KC) H&E X400

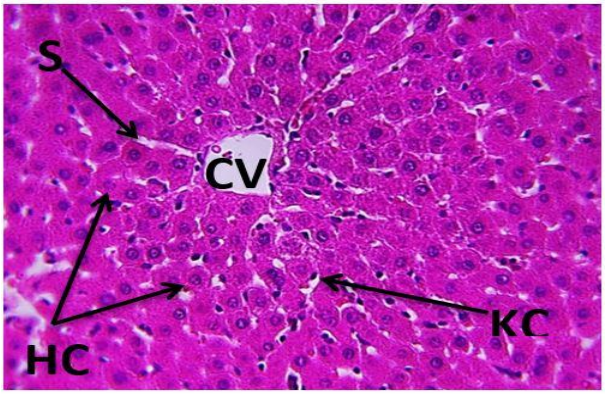

Figure 9. Liver of 5 mg Bisphenol and 400 mg MOLP group show central vein (CV), hepatocytes (HC), sinusoids (S), and Kupffer cells (KC) (H&E ×400)

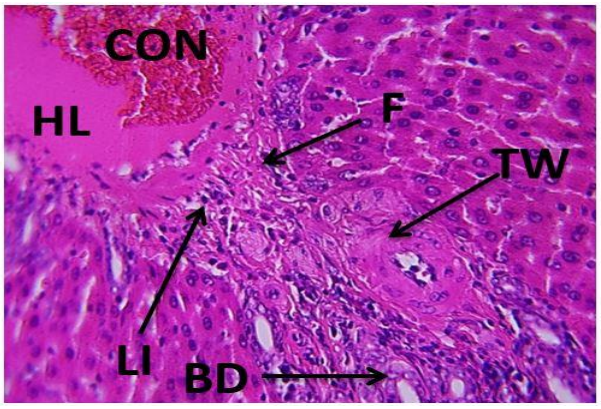

Figure 10. liver of 10mg Bis group show thickening wall (TW) of central vein with congestion (CON), sclerosis of bile duct (BD) that surrounded by fibrocytes (F) and lymphocytes infiltration (LI) with hemolysis (HL) H&E X400.

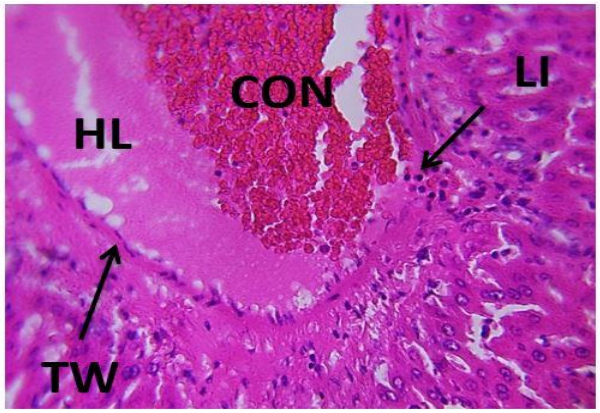

Figure 11. Liver of 10 mg Bisphenol and 100 mg MOLP group show the thickening wall (TW) of central vein with congestion (CON), and lymphocytes infiltration (LI) with hemolysis (HL) (H&E ×400)

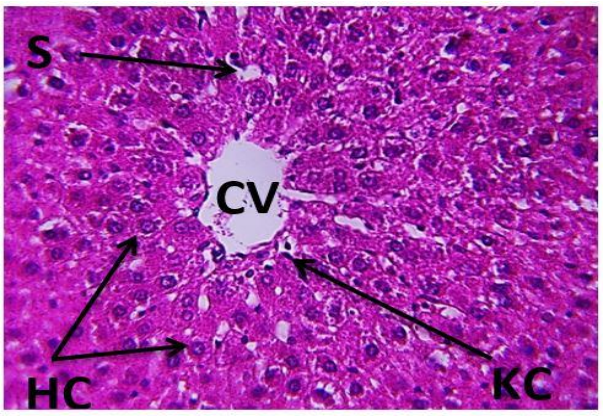

Figure 13. Liver of 10mg Bis & 300mg MOLP group show central vein (CV), hepatocytes (HC), sinusoids (S) and Kupffer cells (KC) H&E X400

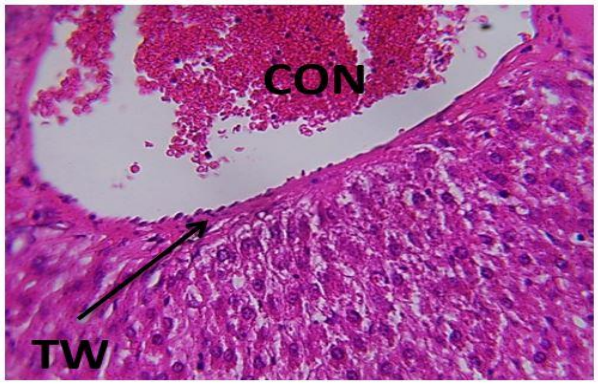

Figure 12. Liver of 10mg Bis & 200mg MOLP group show thickening wall (TW) of central vein with congestion (CON) H&E X400

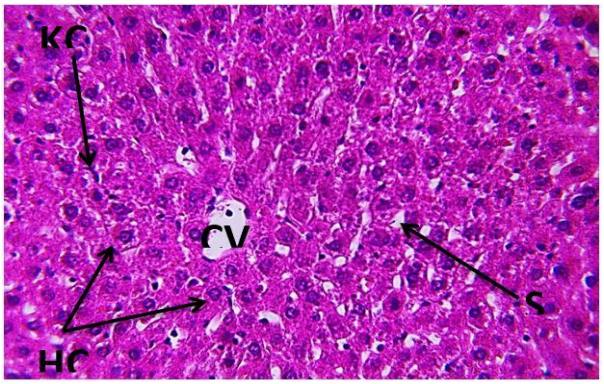

Figure 14. Liver of 10 mg Bisphenol and 400 mg MOLP group show central vein (CV), hepatocytes (HC), sinusoids (S), and Kupffer cells (KC) (H&E ×400)

4. Discussion

Severe reactions (e.g., damage to cellular proteins, lipids, and nucleic acid) are observed today due to the overproduction and consumption of BPA (9, 10). The therapeutic effects of the *M. oleifera* in hepatorenal, cardiovascular, hematological, and gastrointestinal disorders, as well as viral and bacterial infections, rheumatism disease, and hepatic glutathione restoration, were also evaluated (19-21). The current results of the liver enzyme levels showed significant ($P \leq 0.05$) differences, where the liver enzymes levels showed a significant ($P \leq 0.05$) elevation in the groups of BPA (5 mg and 10 mg), compared to the control group. The current findings are consistent with the results of Mohammed and Thalij (25) that indicated the effect of BPA at a concentration of 5 and 10 mg/kg, which led to a significant increase ($P<0.05$) in ALT, AST, and ALP levels for all groups of animals fed on BPA and drinking water, where the values of ALT, AST, and ALP activities reached 38 and 51, 48 and 55, as well as 148 and 155 IU/L, respectively, compared to the control group. The results were also in line with the findings of Korkmaz, Ahbab (26) that indicated an increase in the ALT and AST enzyme activities in rats fed on BPA at a concentration of 25 mg/kg.

The occurrence of a change in the activity of liver

enzymes due to the consumption of BPA by animals can be due to the effects of the substance on the metabolic processes in the liver cells and the inhibition of the activity of the enzymes responsible for it, which causes damage to the liver cells and the exit of enzymes outside the cells into the blood. On the other hand, the liver of the BPA groups (5 mg and 10 mg) showed the thickening wall of the central vein with congestion and sclerosis of the bile duct surrounded by fibrocytes and lymphocytes infiltration with hemolysis. Elhamalawy, Eissa (27) reported that the administration of BPA led to various histopathological changes in the liver of mice, including the congestion of blood vessels and vacuolar degeneration of the hepatocytes, which is in agreement with the current findings. Considering the role of the *M. oleifera* extract, the current study showed improvement in the damaged liver tissue and enzymes level. Ganatra, Umang (28) showed that the methanolic extract of *M. oleifera* had a hepatic protective effect that may be due to the quercetin present in the extract of *M. oleifera*. Moreover, *M. oleifera* extract had effects on the AST, ALT, and ALP levels; in addition, it decreased the levels of lipid profile and lipid peroxidation in the rats' liver (29). Other studies also demonstrated that the administration of the extract of *M. oleifera* in mice led to a reduction in the serum levels of ALT, AST, and ALP (30, 31). Based on the present findings, it can be concluded that liver damage and an increase in liver enzymes occur due to the long-term consumption of BPA. Additionally, the therapeutic effect of the *M. oleifera* extract is significant in reducing damage and liver enzyme caused by the toxic effect of BPA.

Authors' Contribution

Study concept and design: A. I. S.
Acquisition of data: H. M. S.
Analysis and interpretation of data: A. S. K.
Drafting of the manuscript: H. S. A.
Critical revision of the manuscript for important intellectual content: A. I. S.
Statistical analysis: A. I. S.
Administrative, technical, and material support: A. I. S.

Ethics

All the study experiments were approved by the Ethics Committee of the College of Agriculture, Al-Hawija, University of Kirkuk, and College of Agriculture, Tikrit University, Tikrit, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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