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Ganoderma Lucidum Effect on Hepato-Nephrotoxicity Induced by Trihalomethanes in Wistar Albino Rats

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Abstract

Ganoderma lucidum (G.L) is a well-known mushroom that is used extensively nowadays to treat or prevent a variety of diseases, such as heart disease, liver dysfunction, cancer and kidney failure due to its excellent antioxidant activities. Therefore, the objective of present investigation was to evaluate whether G.L could mitigate oxidative stress and avert hepatorenal injury induced by trihalomethanes (THMs) in adult male Wistar albino rats. Forty male Wister rats (130 ± 15 g) were allocated into 4 equal groups (10 rats/group) as follows: Group (1) rats kept as control, whereas group (2) rats received G.L (600mg / kg B.W daily) orally by stomach tube; group (3) rats were intoxicated with THMs (16.17mg / kg B.W daily, injected i.p); group (4) rats were treated with THMs along with G.L for 30 days. After one month of the trial, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (T.P) and albumin (Alb), triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL), creatinine (Cr), urea (Ur), uric acid (UA) and blood urea nitrogen (BUN) were estimated in blood sera. Meanwhile, oxidative stress markers including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reduced content (GSH) and malondialdehyde (MDA) were performed in liver and kidney tissue homogenates. The results of this study declared that THMs declined the levels of Alb, T.P, HDL, renal and hepatic antioxidants (SOD, GPx, CAT and GSH) while raising the levels of ALT and AST, TC, TGs and LDL, kidney function markers, as well as MDA. Administration of G.L to THMs-intoxicated rats reversed the effect of THMs, prevented oxidative stress and provided strong antioxidant protection against THMs induced hepato-renal toxicity.

Keywords: Oxidative stress, Ganoderma lucidum, antioxidants, THMs, Hepatorenal toxicity, Rats

1. Introduction

Chlorination is a widely used technique in many nations to guarantee the safety of their drinking water [1, 2]. It has been successfully applied for the purpose of cleaning drinking water for more than a century [3]. The best and most common method of disinfecting drinking water is chlorine treatment, which is also the least expensive, safest, easiest to use, and least likely to make the water taste bad [4]. However, chlorination of water can initiate the generation of disinfection by-products (DBPs) including trihalomethanes (THMs) which found to be toxic and led to a widespread public health problem on animals and humans [2, 5] including genotoxicity; mutagenicity and cancers as bladder and colon cancer [6].

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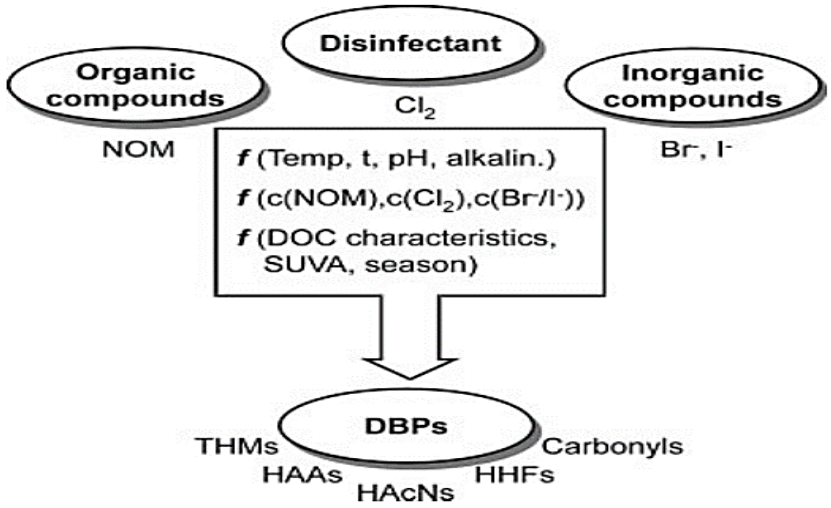

Figure 1: General creation of DBPs via the disinfectants reactions with the constituents of water [7].

THMs are created when naturally occurring organic materials in water, including decomposing vegetation, combines with chlorine [5] as shown in **Figure 1**. Chlorination process usually results in the presence of the following four THMs: tribromomethane (bromoform, TBM), dibromochloromethane (DBCM), bromodichloromethane (BDCM), and trichloromethane (chloroform, TCM) [8]. According to several studies, these substances have been linked to cytotoxicity, genotoxicity, congenital heart abnormalities, growth retardation, cancer, congenital anomalies in fetal during pregnancy and spontaneous abortion [2, 5, 6, 9].

Former studies revealed that THMs are metabolized mainly in the liver, that is the main target organ for human THM exposure, in which THMs compounds are conjugated then transported to the kidney [9, 10]. After that, a small amount is expelled in urine and the rest is accumulated in various body organs. The biotransformation of THM into highly reactive dihalomethyl radicals is likely the pathway by which THM induce cytotoxicity [10]. This process enhances the formation of reactive oxygen species (ROS), which causes lipid peroxidation and cytotoxicity. This could result in necrotic or apoptotic processes that kill cells, or it could set off the development of cancer if genetic mutations are permitted to accumulate [11].

Herbs and medicinal plants are nowadays utilized to promote health through a synergistic reaction, instead of synthetic medications [12]. The potential of medicinal herbs to promote health and treat specific symptoms is drawing more and more attention to their use globally these days because they are less toxic and have no side effects [13]. Among these medicinal plants are reishi mushrooms [14].Chinese physicians have acknowledged reishi mushrooms (Ganoderma lucidum, G.L) as a beneficial treatment for more than 4,000 years. "Spiritual potency" is what its Chinese name (Lingzhi) signifies. The Chinese refer to reishi mushrooms as the "Medicine of Kings" and claim that consuming them a lengthy period of time will give rise to a strong, healthy body and a long life [12, 15].

Ganoderma is a hard-fruiting plant with white roots that degrade wood. Polysaccharides, polypeptides, triterpenes, proteins, lipids, alkaloids, coumarins, lactones, and glutamic acid trace elements are among its active constituents [16]. These active constituents were found to have pharmacological actions that include hepatoprotective, anti-angiogenic, anti-inflammatory, antibacterial, anticancer, antioxidant and antiandrogenic properties [15, 17]. Thus, G.L was extensively utilized in various applications in traditional Chinese medicine, including treatment of cancer, leukopenia, chronic bronchitis, hyperlipidemia, hepatitis, neurasthenia, hypertension, and inflammation [15, 16]. That's what motivated us to perform this study, which looked at the biological effects of G.L on rats 'livers and kidneys injuries induced by THMs as an experimental model.

2. Materials and Methods

2.1. Chemicals

THMs ampule and G.L powder were purchased from Sigma-Aldrich (USA). Kits utilized for determination of liver enzymes (ALT and AST,) and proteins (Alb and T.P), kidney function parameters (Cr, Ur, UA and BUN) and lipid profile markers (TGs, TC, LDL and HDL) were obtained from Biodiagnostic Company, Giza, Egypt. ELISA kits utilized for determination of oxidative markers were purchased from Life Span Biosciences Inc., USA.

2.2. Animals utilized in the experiment

Forty adults male *Wistar albino* rats (weighing 130 ±15 g in average) were bought and raised at the Faculty of Veterinary Medicine's animal house at Suez Canal University. They spent 7 days as an acclimatization period in laboratory conditions in 8 cages (each group was represented by two cages). Water and standard food were provided to the animals on a regular basis, and they were kept in a normal environment at 25 ± 2 °C. All treatments and handling were approved by the animal use ethical committee of Suez Canal University, Faculty of Science. The approval code number of the committee is REC 70/2022.

2.3. Experimental design

Rats were randomly and equally divided into 4 groups once adapted (10 rats/group). Group (1) fed on normal diet as *ad libitum* and received normal tap water and injected with olive oil (vehicle) while group (2) rats received 600mg/kg B.W of G.L orally by tubulation every day as illustrated by Dabdoub, et al. [14]. Group (3) rats were intraperitoneally injected with THMs (16.17mg/kg B.W) daily according to Abd El-Halim, et al. [9]. In group (4) rats were treated with THMs and G.L for 30 days. G.L was administered one hour before THMs injection, and the experimental period lasted one month.

2.4. Sampling

At the end of the trial, blood samples were taken from the retro-orbital plexus of the rats that had fasted overnight after they had been slightly sedated by diethyl ether. Samples were centrifuged for 15 minutes at 3000 rpm after being allowed to clot at room temperature. The serum was separated and stored at -20 °C in sterile stoppered vials until the biochemical tests. Following a thorough anesthesia, the animals were sacrificed by cervical dislocation, liver and kidney tissues were removed, and they were bathed in ice-cold saline. Using Tissue Master TM125 (Omni International, USA), portions were quickly frozen with liquid nitrogen and homogenized in potassium phosphate buffer (pH 7.4). In order to test oxidative stress, the tissue homogenates were centrifuged for 10 minutes at 3000 rpm, and the clear supernatants were kept at -80 °C.

2.5. Biochemical parameters bioassay

The markers of liver enzymes (ALT and AST), protein measurements (Alb and T.P), the activities of kidney functions (Cr, UA, Ur and BUN) and lipid profile (TC, TGs, LDL and HDL were assayed in the obtained serum samples by using colorimetric methods following the kit manufacturer's instructions.

2.6. Biochemical assays in tissues homogenates:

2.6.1. Markers of oxidative stress

The markers were measured in the supernatants of hepatic and kidney tissue homogenates. The measurements of MDA (nmol/g) and the content of GSH (mg/g) were analyzed in accordance with the method illustrated by Ohkawa, et al. [18] and Beutler, et al. [19], respectively. The enzyme activities of CAT (IU/g), SOD (IU/g), and GPx (IU/g) were measured using the techniques recommended by Aebi [20], Nishikimi, et al. [21], and Paglia and Valentine [22], respectively.

2.7. Statistical analysis

The data was presented as mean ± SE (n = 10) and analyzed using one-way ANOVA followed by the Duncan multiple tests in SPSS version 20 (SPSS Inc., Chicago). P value less than 0.05 was taken to be statistically significant.

3. Results

3.1 Effect of G.L on liver enzymes and proteins markers

The current data declared that exposure to THMs significantly ($P \leq 0.05$) increased ALT and AST levels, while Alb and T.P levels were significantly ($P \leq 0.05$) decreased in comparison with the control group. Meanwhile, G.L co-administration led to a significant ($P \leq 0.05$) amelioration in liver enzymes and protein levels compared to the THMs treated group and comparable to control group as presented in **Table (1)**.

Table (1) :*Ganoderma lucidum* (G.L) effect on measurements of liver enzymes and proteins in sera of male rats influenced by trihalomethanes (THMs).

Groups	Parameters			
	ALT (U/l)	AST (U/l)	Alb (mg/dl)	T.P (mg/dl)
Control	40.45 ± 0.11^{c}	56.37 ± 0.09^{c}	5.40 ± 0.10^{a}	9.33 ± 0.94^{a}
G.L	40.56 ± 0.23^{c}	56.40 ± 0.06^{c}	5.33 ± 0.07^{a}	9.57 ± 0.15^{a}
THMs	113.53 ± 0.04^{a}	124.37 ± 0.92^{a}	1.90 ± 0.05^{c}	5.40 ± 0.16^{c}
THMs+G.L	54.6 ± 0.21^{b}	60.53 ± 0.09^{b}	3.77 ± 0.09^{b}	7.60 ± 0.06^{b}

Data were presented as mean ± SE. Values in the same column with distinct superscript letters are statistically significant at $P \leq 0.05$.

3.2 Effect of G.L on kidney function markers

The results expressed in **Table (2)** revealed that THMs-intoxication significantly ($P \leq 0.05$) raised the Cr, Ur, BUN and UA levels compared to the control group. Conversely, G.L supplementation to the THMs treated group significantly ($P \leq 0.05$) lowered Cr, Ur, BUN and UA levels compared to the THMs treated group.

Table (2) :*Ganoderma lucidum* (G.L) effect on kidney function values in sera of male rats influenced by trihalomethanes (THMs).

Groups	Parameters			
	Cr (mg/dl)	Ur (mg/dl)	BUN (mg/dl)	UA (mg/dl)
Control	1.11 ± 0.05^{c}	23.57 ± 0.05^{c}	5.37 ± 0.05^{c}	3.18 ± 0.04^{c}
G.L	1.03 ± 0.03^{c}	23.34 ± 0.03^{c}	5.85 ± 0.09^{c}	3.84 ± 0.05^{c}
THMs	1.57 ± 0.06^{a}	38.63 ± 0.03^{a}	10.22 ± 0.04^{a}	5.29 ± 0.10^{a}
THMs + G.L	1.26 ± 0.03^{b}	30.28 ± 0.02^{b}	7.75 ± 0.03^{b}	4.79 ± 0.05^{b}

Data were presented as mean ± SE. Values in the same column with distinct superscript letters are statistically significant at $P \leq 0.05$.

3.3. Effect of G.L on Lipid profile assays

The data given in **Table (3)** revealed that THMs-intoxicated rats exhibited a significant ($P \leq 0.05$) elevation in TC, TGs and LDH levels, whereas HDL level was reduced significantly ($P \leq 0.05$) in sera as compared to the control group. Administration of G.L to the THMs treated rats significantly ($P \leq 0.05$) reversed the impact of THMs when compared to the THMs treated group.

Table (3) :*Ganoderma lucidum* (G.L) effect on measurements of lipid profile in sera of male rats influenced by trihalomethanes (THMs).

Groups	Parameters			
	TC (mg/dl)	TGs (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	96.47 ± 0.03^{c}	102.49 ± 0.05^{c}	20.37 ± 0.12^{a}	45.60 ± 0.15^{c}
G.L	96.53 ± 0.14^{c}	102.80 ± 0.06^{c}	20.57 ± 0.09^{a}	44.98 ± 0.19^{c}
THMs	202.47 ± 0.09^{a}	240.54 ± 0.23^{a}	9.53 ± 0.15^{a}	166.60 ± 0.18^{a}
THMs+ G.L	113.43 ± 0.03^{b}	153.53 ± 0.15^{b}	15.50 ± 0.06^{b}	58.67 ± 0.14^{b}

Data were presented as mean ± SE. Values in the same column with distinct superscript letters are statistically significant at $P \leq 0.05$.

3.4. Effect of G.L on malondialdehyde and antioxidants in hepatic and renal tissues

The present results expressed in **Table (4)** indicated that rats injected by THMs showed a significant ($P \leq 0.05$) raised MDA levels, however SOD, GPx, CAT and GSH levels were significantly ($P \leq 0.05$) lowered as compared to control group in both tissues. Conversely, G.L co-administration caused a significant ($P \leq 0.05$) ameliorating in MDA and antioxidant levels compared to the THMs intoxicated group.

Table (4) :*Ganoderma lucidum* (G.L) effect on malondialdehyde and antioxidants levels in hepatic and renal tissues of male rats influenced by trihalomethanes (THMs).

Parameters		Experimental groups			
		Control	**G.L**	**THMs**	**THMs+ G.L**
Liver	**MDA (nmol/g)**	96.40 ± 0.06^{c}	96.53 ± 0.14^{c}	132.50 ± 0.16^{a}	109.40 ± 0.12^{b}
	GSH (mg/g)	145.53 ± 0.18^{a}	145.40 ± 0.15^{a}	71.53 ± 0.15^{c}	126.33 ± 0.09^{b}
	SOD (IU/g)	42.54 ± 0.11^{a}	42.63 ± 0.18^{a}	25.47 ± 0.07^{c}	39.50 ± 0.06^{b}
	CAT (IU/g)	78.53 ± 0.19^{a}	78.60 ± 0.06^{a}	30.27 ± 0.12^{c}	62.43 ± 0.03^{b}
	GPx (IU/g)	72.50 ± 0.56^{a}	72.47 ± 0.07^{a}	32.50 ± 0.58^{c}	64.43 ± 0.19^{b}
Kidney	**MDA (nmol/g)**	80.53 ± 0.19^{c}	80.43 ± 0.18^{c}	113.54 ± 0.05^{a}	88.43 ± 0.09^{b}
	GSH (mg/g)	98.50 ± 0.05^{a}	97.78 ± 0.09^{a}	48.60 ± 0.90^{c}	85.51 ± 0.09^{b}
	SOD (IU/g)	96.35 ± 0.08^{a}	96.55 ± 0.19^{a}	58.41 ± 0.18^{c}	93.43 ± 0.12^{b}
	CAT (IU/g)	69.51 ± 0.12^{a}	69.44 ± 0.04^{a}	34.76 ± 0.13^{c}	67.53 ± 0.18^{b}
	GPx (IU/g)	38.41 ± 0.12^{a}	38.73 ± 0.12^{a}	22.53 ± 0.15^{c}	31.50 ± 0.20^{b}

Data were presented as mean ± SE. Values in the same row with distinct superscript letters are statistically significant at $P \leq 0.05$.

4. Discussion

The present research focused on the possible protective and therapeutic role of G.L on oxidative damage caused by THMs, which induces hepato-nephrotoxicity. Liver function tests (LFTs) include AST and ALT are the two most valid commonly used biomarkers for liver damage assessment. ALT and AST are both essential enzymes in gluconeogenesis, with ALT being more specific for the liver whereas AST can be present in a range of organs. [23]. Elevated serum ALT results from hepatocellular injury caused by damaging the hepatic cell membranes and subsequent release of the enzymes into extracellular space. Because AST is found in more organs than ALT, it is less specific for detecting liver impairment. A rise in mitochondrial AST in blood is strongly predictive of tissue necrosis, chronic liver disease and myocardial infarction. [24]. The mitochondrial version of the isoenzymes contributes more than 80% of the liver's AST activity, whereas the cytoplasmic form of AST contributes to blood circulating AST. AST is notably elevated in patients with cirrhosis of the liver [25].

In this study, results indicated that THM toxication elevated ALT and AST levels in group (3) suggesting that THM can induce hepatotoxicity. This result may be due to the hepatic oxidative stress caused by THMs which disrupts integrity of hepatocytes that triggered the outflow of AST and ALT from dented hepatic cells into blood plasma. These outcomes were compatible with Burch, et al. [10] and Faustino-Rocha,

et al. [26] who reported that oral administration of THMs compounds (117 mg/kg/day) substantially raised ALT level.

The co-administration of G.L to rats intoxicated with THMs in group (4) improves hepatic levels, suggesting that G.L has hepatoprotective properties against THMs intoxication. This amelioration may be due to G.L's antioxidant activities and therapeutic value. These findings were consistent with Ahmad, et al. [27] and Dabdoub , et al. [14] who revealed that oral administrating of G.L aqueous extract (600 mg/kg) for 12 weeks overcome the toxic impacts of carbon tetrachloride and substantially raised hepatic markers levels.

The present data clarified that THMs intoxicated rats in group (3) showed substantial reduction in T.P and Alb as compared to control groups. The reduction in T.P and Alb might be attributed to hypoproteinemia and coagulation induced by THMs intoxication. Due to the disruption of the structure and the function of liver induced by THMs intoxication [10] since liver is the chief organ responsible for the plasma protein biosynthesis [25]. In addition to the down regulation resulted in the blood oncotic pressure, leading to loss of fluid from the blood vessels, or intravascular compartment, to the interstitial tissues, causing edema [28].

Meanwhile, administrating G.L along with THMs in group (4) caused upregulation in T.P and Alb levels. This elevation is attributed to the retinoid's antioxidant and hepatoprotective properties of G.L that have been shown in numerous research. G.L promotes hepatic cells regeneration. Thus it preserves liver function in the biosynthesis of proteins and strengthens its cellular membrane while decreasing enzyme leakage. This result was in agreement with Sayed Ahmed, et al. [29] and Lin and Lin [30].

This study indicated that rats intoxicated with THMs showed substantial upregulation in TC, T.G and LDL levels, whereas HDL levels declined in group (3). This might be attributed to liver injury resulting from THM toxic effect. As elevated THM levels cause their molecule to aggregate in the liver, disrupting lipid metabolism and raising the lipid profile markers [10]. Liver damage also comes along with decreased glucose and tolerance insulin responsiveness. then it leads to increase free F.A, cholesterol, T.G lipoproteins (LDL) and decrease HDL [31]. These outcomes were in harmony with Anand and Mehendale [32].

The administration of G.L reversed the impacts of THMs and improved lipid profile levels. This might be attributed to the hypocholesterolemic properties of G.L and the presence of triterpenes in its active components that lower the blood cholesterol and prevent hyperlipidemia [16]. This finding was compatible with Tong, et al. [16] who reported that co-administrating of G.L (50, 100, 150 mg/kg/day) for 4 weeks prevents hyperlipidemia and reduces lipid metabolic diseases by manipulating certain gut microbes in a dose dependent manner in high-fat diet rats.

The kidneys perform an important function in the excretion of waste and poisons. including Ur, BUN, Cr and UA [14]. Evaluation of renal functions is vital in managing any kind of kidney disease that affects its functions. [33]. In this study, results indicated that THM toxication elevated creatinine, urea and BUN levels in group (3) suggesting that THM can induce nephrotoxicity, causing impaired renal function and hyperuricemia. This result might be attributed to the renal oxidative stress caused by THMs, which can damage the basement membrane of the glomerular cells and change the glomerular and tubular cell processes.[34]. Moreover, elevated uric acid level in chronic kidney disease (CKD) may lead to tubular injury and intra-renal inflammation [35]. These findings were compatible with Kroll, et al. [34].

In contrary, co-administrating G.L to rats intoxicated with THMs in group (4) improves the levels of renal functions parameters, suggesting that G.L has nephroprotective properties against THMs intoxication. This improvement may be due to hexadecanoic acid’s antioxidant activities and therapeutic properties of G.L that promotes renal cells regeneration [33, 36]. These outcomes were in harmony with Meneses, et al. [33] and Hu, et al. [37].

Oxidative stress is caused by the excessive generation of ROS, such as hydrogen peroxide, superoxide, and hydroxyl radicals, due to the imbalance between antioxidants and free radicals in the body [26]. Our results suggested that THMs toxication may contribute to hepatic and renal oxidative damage that consequently resulted in hepato-renal toxicity. This was manifested by raising the hepatic MDA concentration, and decreasing the antioxidants of the tissue, which includes SOD, GPx, CAT, and GSH content. Lipid peroxidation in hepatic and renal cellular membranes may be the cause of the observed increase in liver and renal oxidative stress, resulting in their damage. These were consistent with Faustino-Rocha, et al. [26] who indicated that THM-resulted in cellular injury due to the excessive production of ROS and free radicals that damage many biomolecules and cause a wide range of molecular and cellular effects, thus inducing lipid peroxidation that resulted in cytotoxicity.

The co-administration of G. L ameliorated the THMs impacts in renal and liver tissues by upregulating GSH content and SOD, GPx and CAT activities and downregulating MDA levels. It is supposed that G.L has antioxidant action and can neutralize oxygen and peroxyl radicals leading to a low generation of MDA level [36]. These results matches Dabdoub, et al. [14] who found that oral administrating of G.L aqueous extract (600mg/kg) for 12 weeks overcome the

oxidative damage induced via CCl_4 toxicity in liver and kidney that manifested by the amelioration of antioxidant markers (SOD, GSH and CAT) while decreasing the levels of MDA. Also, Susilo, et al. [38] proved that G.L have antioxidant activity against CCl_4 hepatotoxicity induced in mice that was evidenced by lowering MDA concentration and upregulating the levels of antioxidant enzymes including SOD and CAT.

5. Conclusion

In summary, these results clarified that G.L produces a substantial protective effect against hepatic and renal toxicity by improving the liver enzymes, kidney functions as well as the hepatic and renal oxidative damage that evidenced via lowering MDA levels and increasing antioxidants (CAT, SOD, GSH and GPx) levels. As a result, G.L could lower oxidative stress induced by THMs in hepatic and renal tissue.

6. Abbreviations

G.L: *Ganoderma lucidum*; THMs: trihalomethanes; B.W: body weight; i.p: intraperitoneal; DBPs: Disinfection byproducts; DBCM: dibromochloromethane; BDCM: bromodichloromethane; BUN: blood urea nitrogen; GPX: Glutathione Peroxidase; CAT: Catalase; SOD: Superoxide Dismutase; GSH: reduced Glutathione.

7. Conflicts of Interest

The authors declare that there are no conflicts of interest

8. Formatting of funding sources

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