BIOCHEMICAL AND HISTOLOGICAL STUDY ON BERBERINE-CHITOSAN NANOPARTICLES EFFECTS ON IMPROVEMENT OF THE INDUCED LIVER AND KIDNEY DAMAGE IN MALE RATS

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Abstract: In this study, we examined the berberine-chitosan nanoparticles (BCNP) action as a natural polymer used to enhance liver and kidney damage induced by phenylhydrazine in male rats. Healthy 20 male Sprague-Dawley rats weighed 150 - 200 g were used in this study and the animals were randomly divided into four groups, G1: (CON), G2: chitosan (CHS), G3: berberine (BER), and G4: berberine-chitosan NPs (BCNPs). Serum ALT, AST, TP, and TB for assessment of liver damage, and creatinine (CRI) and urea (UR) were measured to investigate kidney damage. The administration of experimental rats with BCNPs done by the current study BCNPs with the dose of 0.5 mg/Kg B.W. will be given protection from hepatotoxic and kidney damage action of phenylhydrazine. Liver sections revealed moderate improvement of the hepatic artery, portal vein, and bile duct and reorganization of hepatic cords. Kidney sections illustrated also moderate improvement of tubular necrosis. In conclusion, the usage of BCNPs as a natural polymer with moderated modifications has greatly improved the curative effect of pure BER and CHS to protect the liver and kidneys against toxicity induced by phenylhydrazine that may be by a reduction in lipid peroxidation and enhancement of the main markers of liver and kidney.

Keywords: Liver and kidney damage, Berberine, Chitosan, drug delivery, NPs

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INTRODUCTION

The organ that is responsible for many critical functions within the body has been called the liver (1). The last studies suggested that the liver disease is a broad term that covers all the potential problems and declines its functions (2-5). The presence of kidney damage has been called chronic kidney disease (CKD) (6,7). The molecular formula of $C_{20}H_{19}NO_5$ and molecular weight of 353 has been called berberine (BER). BER has been reported that used to treat gastrointestinal infections in China (8). BER is an isoquinoline alkaloid, frequently utilized as an antidiarrheal, got from the rhizome of Coptis chinensis ("Huang-Lian" in Chinese) of the Ranunculaceae family (9).The one amongst the foremost standard materials within the field of drug delivery has been known as chitosan (CHS) and it's the foremost applied of the natural polymers (10). CHS is offered in an exceedingly sort of forms that chiefly dissent within the relative molecular mass and degree of deacetylation (11). The applications of CHS within the field of drug delivery are principally that specialize in the assembly of carriers that improve the encapsulated potency (12). The applications of chitosan-based NP are investigated in several studies for the tissue toxicity (13-15). The purpose of present study to examine the BER-CHS nanoparticles (BCNPs) action as natural polymer by used to improvement of liver and kidney damage induced by phenylhydrazine in male rats.

MATERIALS AND METHODS

Materials:

This study was conducted during the period December- March 2021 in the physiology department of veterinary medicine of AL-Qasim Green University and with a corporation with the educational veterinary hospital in Babylon city. Chitosan was purchased from MCE/ USA, while berberine was obtained from BIOCHEM/ China and other chemicals and kits provided by the biochemistry laboratory of above department.

Experimental Design and animal distribution:
Sixteen healthy male of anomaly rats weighed 150- two hundred g were purchased from the animal house of college of medicine, University of Kufa, and used for this study. The associate animals were indiscriminately divided into four teams of 4 rats in every when an adjustment amount of time period. The diet, drink, and temperature has been approved depending on protocol that suggested by the Institutional Animal Ethics Committee of Al-Qasim Green University and the dose was 0.5 mg/ kg W.B.) for all.

- G1 (4 rats: CON): Animals injected phenylhydrazine (1 ml/kg body weight/day) for one week (induced toxicity) and 5ml/kg body weight/day saline solution subcutaneously for 8 weeks.
- G2 (4 rats: CHS): Animals injected phenylhydrazine (1 ml/kg body weight/day) for one week (induced toxicity) and 5ml/kg body weight/day CHN solution for 8 weeks.
- G3 (4 rats: BER): Animals injected phenyl hydrazine (1 ml/kg body weight/day) for one week (induced toxicity) and 5ml/kg body weight/day BER solution for 8 weeks.
- G4 (4 rats: BCNPs): Animals injected phenylhydrazine (1 ml/kg body weight/day) for one week (induced toxicity) and 5ml/kg body weight/day BCNPs solution for 8 weeks.

Preparation of BCNPs:
BCNPs were prepared by protocol that described by Grenha A , 2012(16). Briefly, BER and CHS was dissolved in HCL and acetic acid respectively. Both of them solutions mixed under stirring drop by drop. By washing with water at three times to remove extra BER mixed with the surface of prepared NPs.

Characterization of BCNPs:

PS, PDI, and ZP Measurement:
Zeta sizer with DLS technique by Nano-ZS, Malvern Instruments, Malvern, UK were used to investigated the PS, PDI, and ZP of BCNPs.

Estimation of Encapsulation Efficiency (EE%) and Drug-Loading (DL%):
The EE% and DL% potential of the BCNPs was measured depending on protocol that described by Hamzah HK et al; 2022 (23) according to following:

$$\%EE = X1 - X2/X1 * 100$$

$$\%DL = X1 - X2/X3 * 100$$

where X1 = amount of BER loaded in the BCNPs, X2 = amount of non-entrapped BER, and
X3 = weight of BCNPs

SEM and TEM analysis of BCNPs:
The SEM and TEM analysis were used to assessing of the morphological STATUS of structural size and surface of prepared BCNPs.

FTIR Spectroscopy Analysis:
FTIR spectroscopic study was performed to assessed the spectrum of BER, CHN and BCNPs (about 1 mg) depending on protocol described by Kohli, K et al; 2021 (27).

In Vitro Drug-Release Kinetics of BCNPs:
This performed depending on the protocol suggested by Zadeh; 2022(24) and the BER release from BCNPs formulations has been estimated through progression of time of experiment.

Assessment of ALT and AST:
The levels of ALT and AST activities in serum of rats were determined calorimetrically by using Biolabo diagnostic kits (France) according to manufacture protocol. The absorbance of the assay (A) was read against blank at 540 nm after 5 min. U/ml were used to expressed of activity the ALT and AST with comparing to specific standard curves.

Assessment of total protein and total bilirubin (TP and TB)
Serum TP and TB were performed calorimetrically using Biolabo diagnostic Kits (France) according to the method illustrated in kit. Albumin level was calculated using the following equation: TP and TB levels in mg/dl = concentration of standard TP or TB × Abs A/Abs S.

Determination of serum critinine and urea:
Serum CRI and UR was determined calorimetrically using Biolabo diagnostic Kit (France), according to manufacture protocol.

Histo-pathological assessment
Rats' livers and kidneys were fixed in 10% formalin buffer. The sections of 5–7 μm thick were prepared. For each animal, 2 sections were stained by H&E (Hematoxylin and Eosin) and evaluated for histological criteria including hepatocellular and proximal tubule damage.

RESULTS

Characterization of BCNPs
From the table 1, ZP, PS, PDI, EE% and DL% of BCNPs showing best results in round 3 of nanoparticle synthesis.

Table 1. ZP, PS, PDI, EE% and DL% of BCNPs

ROUND	Organic phas	PS (nm) mean±SD	PDI mean±SD	ZP(mV) mean±SD	EE% mean±SD	DL% mean±SD
1	DMSO	244±23	0.211±0.012	+33±2.2	79±4.7	6.42±1.5
2	DMSO	243±17	0.145±0.013	+33±3.4	77±2.5	7.02±1.3
3	DMSO	241±12	0.323±0.021	+31±4.6	80±3.6	7.09±1.4

Figure 1 and 2, showing (a): PS and ZP , (b): SEM of BCNPs analysis that characterization through procedure that showing the morphological structure of nanoparticles with 194 nm as average.

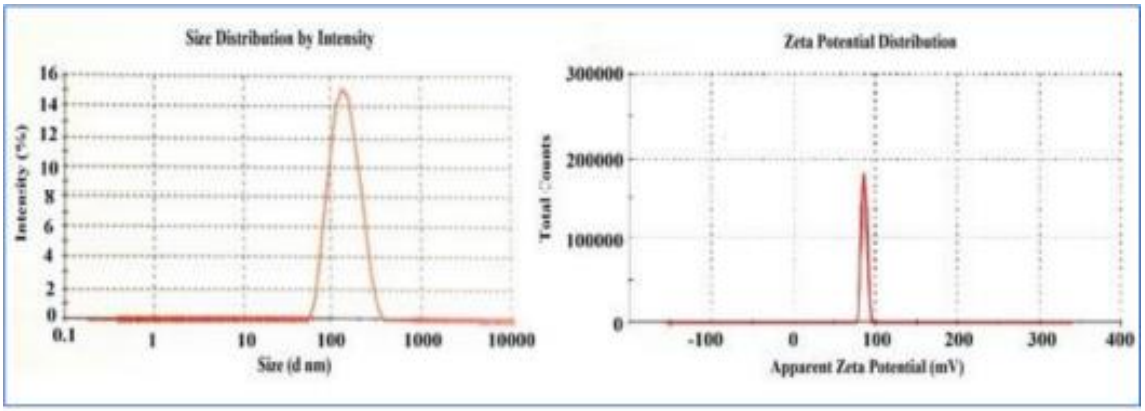


Figure 1. PS and ZP analysis of BCNPs

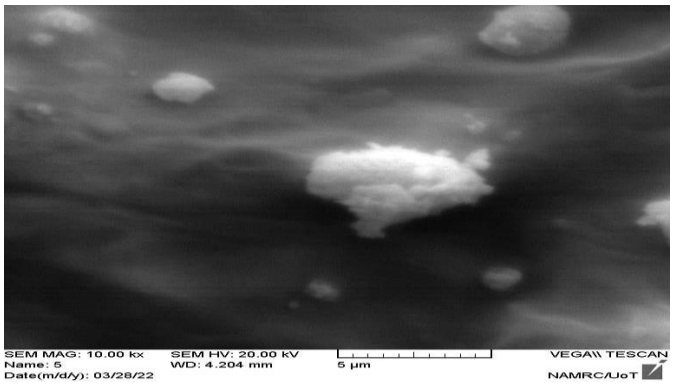
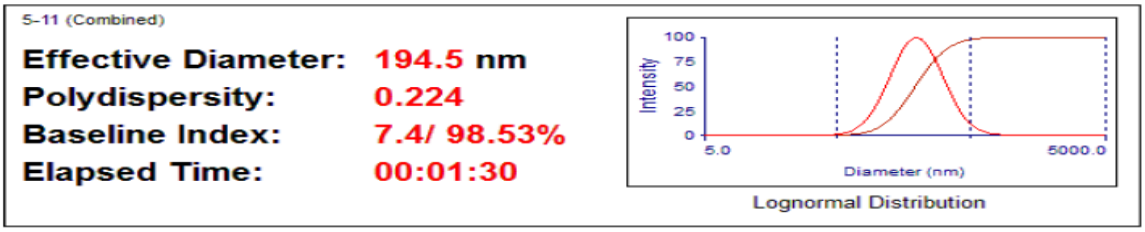


Figure 2. SEM analysis of BCNPs



Run	Eff. Diam. (nm)	Half Width (nm)	Polydispersity	Baseline Index
1	189.7	90.3	0.227	8.6/ 100.00%
2	188.1	90.2	0.230	6.4/ 95.58%
3	205.4	96.9	0.223	7.9/ 100.00%
Mean	194.4	92.5	0.226	7.6/ 98.53%
Std. Error	5.5	2.2	0.002	0.6/ 1.47
Combined	194.5	92.0	0.224	7.4/ 98.53%

Figure 3. (a) Particle size (nm) and (b) Zeta potential (mV) of BCNPs

The berberine release study from BCNPs was carried out with a dialysis membrane bag as shown in figure 4. The release study was conducted on lyophilized BCNPs and was compared to that of free BER and CHS suspension. It was observed that 80% of the BCNPs was released in about 24 h, whereas optimized BER formulation showed an initial release of 25%, followed by a continuous and controlled release of 20 % of CHS in first of 24 h from BCNPs.

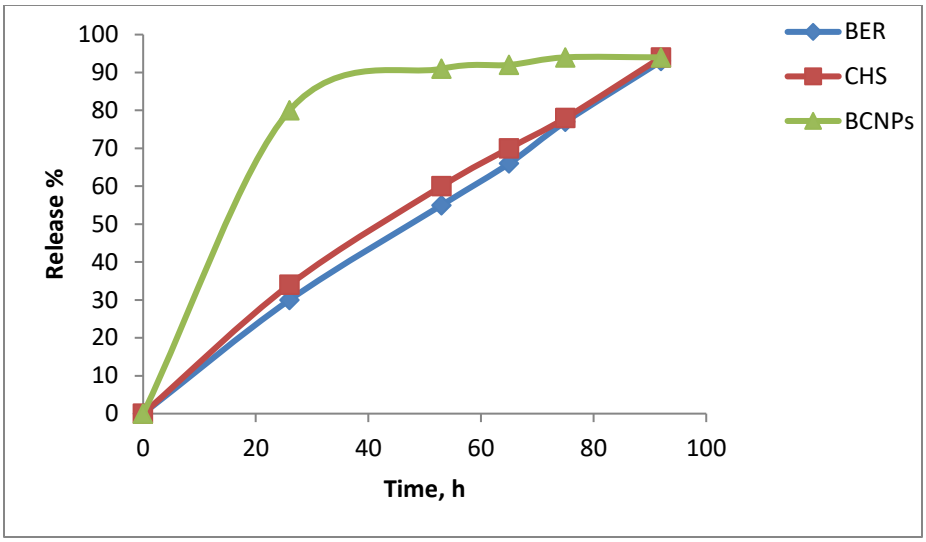


Figure 4. Berberine release percentage curve as BCNPs in vitro study

The results of FT-IR analysis show a methoxyl cluster (peak at two.844 cm^{-1}) of the spectrum of BCNPs and also the peak at one (635 cm^{-1}) and (1569 and 1506 cm^{-1}). No chemical interaction among the BER, CHS, and in BCNPs, as shown in figure 5:

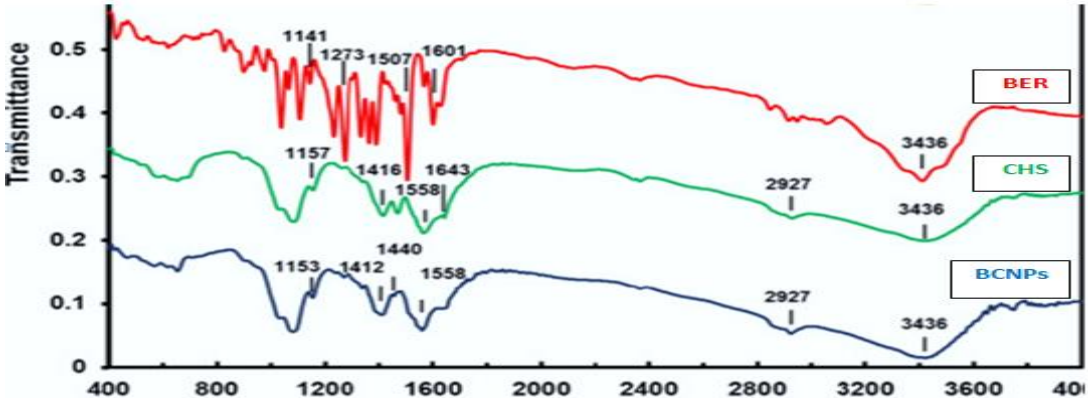


Figure 5: FTIR analysis of BER, CHS, and BCNPs

Table 2, showing the levels of ALT, AST, TP, and TB as liver assessment:

Table 2. ALT, AST, TP, and TB as liver assessment

Parameters	CON	CHS	BER	BCNPs	P-Value
ALT (U/ml)	227.6 ±1.3	144.9 ±7.8	147.7 ±6.5	48.0 ±4.1	< 0.01
AST (U/ml)	194.4 ±2.2	84.6 ±2.7	87.5 ±2.2	64.1 ±1.8	< 0.01
TP (mg/dl)	45.6 ±1.2	25.3 ±1.1	19.2 ±1.7	11.5 ±1.9	< 0.01
TB (mg/dl)	4.79 ±0.012	3.79 ±0.013	3.08±0.008	1.79±0.021	< 0.01

Figures 6, showing the statistically significant differences between study groups in ALT, AST, TP, and TB as administration of BER, CHS, and BCNPs formulations.

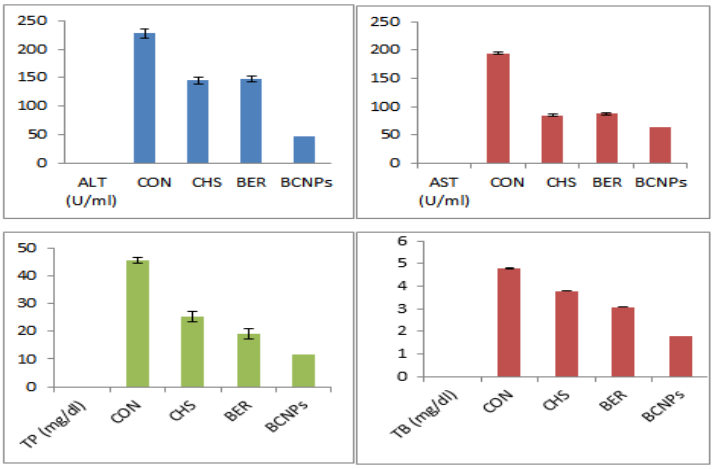


Figure 6: ALT, AST, TP, and TB levels in study groups as liver recovery

Table 3, showing levels of CRI and UR as kidney assessment:

Table 3: CRI and UR as kidney assessment

Parameters	CON	CHS	BER	BCNPs	P-Value
CRI	2.575±0.07	2.245±0.08	2.145±0.09	0.987±0.03	< 0.01
UR	29.49 ± 1.9	25.49 ± 1.8	23.06 ± 1.7	19.86 ± 0.8	< 0.01

Figure 7, showing the significant differences in levels of CRI and UR levels between study groups.

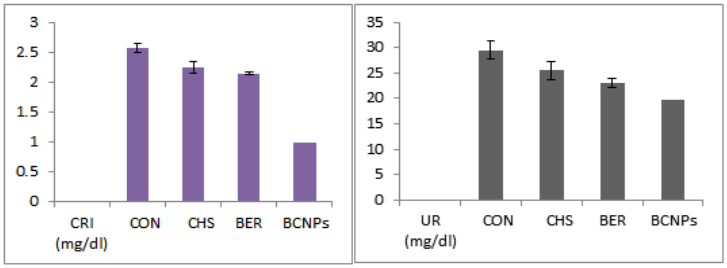


Figure 7: CRI and UR levels in study groups as kidney recovery

The newly formed granulation tissue thickness was significantly increased on end of experiment with Free BER and BCNPs treatments compared to the negative control. BCNPs treatment showed the formation of skin appendages at end of experiment also. Therefore, the regenerated tissue appeared thicker in the BCNPs group. The histological study suggested the protected effect of BCNPs on liver and kidney tissues, as shown in figure 8.

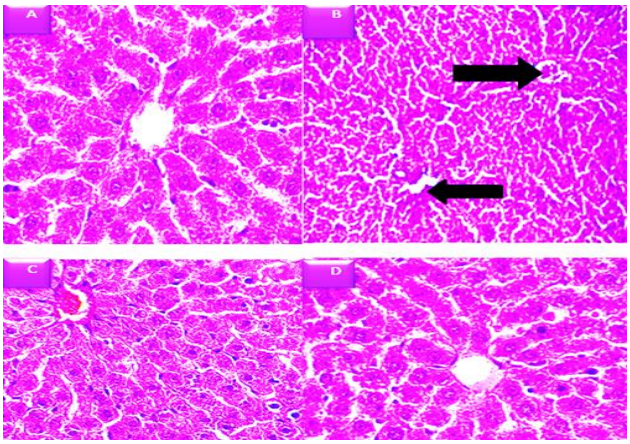


Figure 8. Histological study on liver and kidney tissue treated with BCNPs. Section of liver rats (A; treated with free BER) and (D; treated with BCNPs); Section of kidney rats (B; treated with free BER) and (C treated with BCNPs).

DISCUSSION

The current work was aimed toward the synthesis of chemical compound nanoparticles of BCNPs with chitosan as biopolymers as a result of chitosan is thought to be biocompatible, perishable, and permeation enhancers. it's been found that BER has effective against polygenic disease, metabolic syndrome, high blood pressure, and polycystic ovary (17). For evidenced too sure of hepatotoxicity and kidney damage due to phenylhydrazine results in promoting of necrosis of hepatic cells and increase of intracellular metabolic enzymes, causing tissue malfunction (18-22). The results of liver assessment showing 227.6 ±1.3, 144.9±7.8, 147.7 ±6.5, 48.0 ±4.1 for CON, CHS, BER, and BCNPs respectively. This results indicated that protective action of BCNPs on liver damage and showing recovery for infection. AST, TP and TB showing same effects of ALT with return to normal ranges after 8th weeks of ingestion of rats with BCNPs. This results were agreement with results on rats working by other authors in different region (23-29). The results of kidney assessment showing CRI, 2.575±0.07, 2.245±0.02, 2.45±0.09, and 0.987±0.03 for CON, CHS BER, and BCNPs groups respectively. UR showing same effects of CRI with return to normal ranges after 8th weeks of ingestion of rats with BCNPs. As shown in Table 1, the injection by phenylhydrazine showed a significant ($p < 0.05$) and marked elevation in serum levels of ALT,AST,TP and TB as compared to the negative control group. Treatment with BCNPs at the doses of (0.5 mg/kg) significantly ($p < 0.05$) decreased these liver parameters as compared to the phenyl hydrazine group. It is worth noting that BER and CHS at the dose of (0.5 mg/kg) decreased serum levels of ALT,AST,TP and TB at levels that were not significantly different ($p < 0.05$) from the normal values. As shown in Table 2, the injection with phenylhydrazine showed a significant ($p < 0.05$) and marked elevation in serum levels of CRI and UR as compared to the negative control group. Moreover, phenylhydrazine was found to induce oxidative stress in hepatic tissue leading to accumulation of free radicals and consequently decreased activities of hepatic antioxidant enzymes; SOD, CAT and GPx with increased level of MDA indicating the oxidative damage to the hepatic tissue caused by phenylhydrazine. The hepatoprotective and antioxidant properties of BCNPs are supported by the findings of Panda et al. (30), who found that administration of BER to rats significantly improved all the hepatic toxicity biomarkers in phenyhydrazine-induced liver fibrosis in addition to improve liver histology. This study revealed that liver sections moderate improvement of the hepatic artery, portal vein, and bile duct and reorganization of hepatic cords. Kidney sections illustrated also moderate improvement of tubular necrosis Treatment with BCNPs at the dose of (0.5 mg/kg) significantly ($p < 0.05$) declines these kidney parameters as compared to the phenylhydrazine group, and the. It is correct noting that BER and CHS at both doses (0.5 mg/kg) decreased serum levels of CRI and UR. Phenylhydrazine showing improve of hepatic damage is a well-established model for screening the hepatic-protective meffects of BCNPs. In conclusion, BCNPs found as protective agent against liver and kidney toxicity and damage by Phenylhydrazine in rats.

CONCLUSION

The in vivo study proved that combining CHS and BER in the nanoparticles produces a synergistic effect regarding liver and kidney protection. So the usage of BCNPs as a BER delivery NPs has greatly improved the curative effect of pure berberine and chitosan to protects the liver and kidneys against toxicity induced by phenyl hydrazine by reduction toxicity and enhancement the main markers of liver and kidney. From the overall results of the biochemical and histological examinations, it could be inferred that BCNPs showed the beneficial effect (especially at dose of 0.5 mg/kg Body weight) on liver and renal functions.

Ethical approval: Ethical issues (Animal handling, plagiarism, consent to publication, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) were verified by all authors.

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Conflict of interest : Non.

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Author contributions: Conceptualization, HHK and MEA ;methodology, AGA and HHK.; software, HHK; validation, ZHK, EFH and MEA.; formal analysis, HHK; investigation, HHK; resources, HHK; data curation, SJH; writing—original draft preparation, HHK; writing—review and editing, HHK; visualization, AFF; supervision, EFH; project administration, AFF; funding acquisition, MEA. All authors have read and agreed to the published version of the manuscript.

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