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## Phytochemical and Pharmacological Evaluation of *Lindera communis* Hems/for their Hepatoprotective Activity



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### ABSTRACT

*Lindera communis* Hems is a synonym of *Lindera formosana* Hayata. Belonging to the family of Lauraceae. It includes herbs, shrubs and small trees, *Lindera communis* possess a wide variety of activities. The pericarp contains aromatic oil. The seed oil is used in food and for making soap and machine oil. The branch, leaves and leaves are used medicinally. Hence, the present study was intended to evaluate ethanolic leaf extract of *Lindera communis* for hepatoprotective activity using paracetamol (2g/kg) and D-galactosamine (400mg/kg) induced models. Acute toxicity study and preliminary phytochemical screening were also studied to evaluate the toxicity. No toxicity profile was observed in rats after oral administration of the ethanolic leaf extract at the dose of 5g/kg body weight. The different dose of 200 mg/kg and 400 mg/kg administered with the extract of *Lindera communis* there was significant ( $P < 0.001$ ) reduction in biochemical parameters with respect to control. Phytochemical screening of the plant extract revealed the presence of tannins, alkaloids, flavonoids and saponins, and terpenoids. It can be concluded that the hepatoprotective activity elucidated by *Lindera communis* could be mainly due to the presence of high value of phenolic class of compounds as the major content in the plants.



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## INTRODUCTION

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for ayurvedic, unani, siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Sandhu, D.S et al., 2005; Gupta, M.P et al., 2005). In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and to other plants. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward *et al.*, 1999). The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate (Pang *et al.*, 1992). Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders (Ross *et al.*, 1996). Hence, natural products from medicinal plants need to be investigated by scientific methods for their hepatoprotective activity. The plant *Lindera communis* a synonym of *Lindera formosana* Hayata, belonging to the family of Lauraceae. It includes herbs, shrubs and small trees, The pericarp contains aromatic oil. The seed oil is used in food and for making soap and machine oil. The branchlets and leaves are used medicinally.

## MATERIALS AND METHODS

### Collection, Identification and Authentication of the Plants.

The leaves of *Lindera communis*, were collected from the Malappuram district, Kerala, India, during the month of October 2013. The plant materials were identified and authenticated by Dr. Pradeep Botanist Calicut university, Kozhikode. Voucher specimens were kept in our laboratory for future reference.

### Preparation of extracts

The granulated dried leaves of *Lindera communis* (500 g) was packed in a Soxhlet apparatus and subjected to continuous hot percolation for 8 hrs using 450 ml of ethanol (95 % v/v) as solvent.

The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 68.5 g, 13.72% w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

### **Preliminary phytochemical screening**

The extract was screened qualitatively for the presence of various groups of Phytoconstituents using different chemical tests (Hayden, W.J. et al., 2000; Kokate CK et al., 1986).

### **Procurement of experimental animals**

Animals were selected as per the OECD guidelines. Healthy young and nulliporous, nonpregnant Sprague-Dawley's female Rats weighing from 160-180 mg of 8 – 12 weeks old were selected, because literature survey of LD<sub>50</sub> test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive. Animals were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan Lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.-P.Col/02/1868/26/09/2013/IAEC/JSPC) after scrutinization. The animals received the drug treatments by oral routes.

### **ACUTE TOXICITY STUDIES**

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines. Animals were divided in groups (n=5). Animals were observed individually for 48 hrs after dosing at the first 30 minutes, periodically and during the first 24 hrs, with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. The Ethanolic extract of *Lindera communis* had good margin of safety and did not show any lethal effects on the animals up to the doses of 5000mg/kg. Hence the LD<sub>50</sub> of *Lindera communis* was considered as 5000mg/kg. Studies were carried out with 1/10 of the LD<sub>50</sub> as effective dose 200mg/kg and double the dose of effective dose 400 mg/kg. Additional observations were also

made if the animals continue to display signs of toxicity. Observations included were changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Observations were also made and checked for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Results were tabulated in table no.2

**Experimental procedure:** (Das SK, Roy C. et al., 2012; Darbar S, et al., 2010).

The rats were divided into the five groups each containing 6 rats.

**Group -I:** Control rats, which fed normal diet and water.

**Group -II:** Rats treated with Paracetamol (2g/kg ) for 28 days.

**Group-III:** Rats treated with Silymarin (100 mg/kg) + Paracetamol (2g/kg ) orally once daily for 28 days.

**Group-IV:** Rats treated with LC (200 mg/ kg, i.p.) + Paracetamol (2g/kg ) once daily for 28 days.

**Group-V:** Rats treated with LC (400 mg/ kg, i.p.) + Paracetamol (2g/kg ) once daily for 28 days.

**Experimental procedure:** (Das SK, Roy C. et al., 2012; Darbar S, et al., 2010).

The rats were divided into the five groups each containing 6 rats.

**Group -I:** Control rats, which fed normal diet and water.

**Group -II:** Rats treated with D-Galactosamine (400mg/kg) for 28 days.

**Group-III:** Rats treated with Silymarin (100 mg/kg) + D-Galactosamine (400mg/kg) orally once daily for 28 days.

**Group-IV:** Rats treated with LC (200 mg/ kg, i.p.) + D-Galactosamine (400mg/kg) once daily for 28 days.

**Group-V:** Rats treated with LC (400 mg/ kg, i.p.) + D-Galactosamine (400mg/kg) once daily for 28 days. (Das SK, Roy C et al., 2012; Darbar S et al., 2010).

### 6.1.2 Statistical analysis

The results of various studies were expressed as mean  $\pm$  SEM and analyzed statistically using one way ANOVA followed by dunnettstest to find out the level of significance. Data were considered statistically significant at minimum level of  $p < 0.05$ .

## RESULTS

### Preliminary phytochemical screening

The preliminary phytochemical analysis of fractions of *Lindera communis* shows presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate.

**Table 1. Acute toxicity study of ethanolic extracts of leaves of *Lindera communis* based on OECD guidelines 423**

S. No	Number of animals	Dose in mg/kg	Report
1	3	5mg/kg	No death
2	3	50mg/kg	No death
3	3	300mg/kg	No death
4	3	2000mg/kg	No death
5	3	5000mg/kg	No death

**Table 2. Results of gross behavioral studies in Rats on administration of *Lindera communis***

Observation	Effects								
	Upto 3hrs	3 ½hrs	4 hrs	4 ½hrs	5hrs	5 ½hrs	6hrs	12hrs	24hrs
Gross activity									
Respiration	+	+	+	+	+	+	+	+	+
Writhing	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-	-	-	-
Sense of touch and sound	+	+	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+	+	+
Diarrhoea	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-

**Table 3. Results of the effects of biochemical markers of Paracetamol induced hepatic injury in rats**

S. no	Group/Drug	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
1	Group I- Normal control (NaCl 0.9% w/v)	5ml/kg	51.23 ± 2.31	60.12 ± 1.24	27.17 ± 1.72	1.14 ± 0.13	8.74 ± 0.84
2	Group II- Paracetamol	(2g/kg) *	193.60 ± 1.74*	173.10 ± 1.36*	58.75 ± 1.32*	12.18 ± 0.74*	4.89 ± 1.31*
3	Group III- Silymarin+ Paracetamol	100mg/kg + (2g/kg)**	59.41 ± 1.32**	56.78 ± 2.32**	29.34 ± 1.54**	1.27 ± 0.22**	8.03 ± 0.41**
4	Group IV – LC+Paracetamol	200mg/kg + (2g/kg) **	58.83 ± 1.47**	65.41 ± 1.02**	40.01 ± 1.21**	3.02 ± 0.05**	6.42 ± 1.73**
5	Group V – LC+Paracetamol	400mg/kg + (2g/kg)**	49.81 ± 7.03**	59.38 ± 1.26**	34.76 ± 1.76**	2.81 ± 1.52**	6.91 ± 1.52**

n=6; values were expressed Mean±S.E.M; ; Group II was compared to Group I. Groups III to V were compared to group II. \*p < 0.01 vs. Paracetamol group: significant; \*\* p < 0.001 vs. paracetamol group: highly significant Data were analyzed by One-way ANOVA followed by dunnett's't' test.

**Table 4. Results of the effects of biochemical markers of D-Galactosamine induced hepatic injury in rats**

S. no	Group/Drug	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
1	Group I- Normal control (NaCl 0.9% w/v)	5ml/kg	64.23 ± 1.31	77.52 ± 1.04	19.17 ± 1.35	1.39 ± 1.42	6.98 ± 0.25
2	Group II- D-Galactosamine	(400mg/kg)*	167.70 ± 1.07*	181.02 ± 1.24*	42.83 ± 2.01*	8.31 ± 0.36*	3.66 ± 1.46*
3	Group III- Silymarin+ D-Galactosamine	100mg/kg + (400mg/kg)**	67.74 ± 1.93**	80.21 ± 2.06**	18.64 ± 1.27**	1.43 ± 1.52**	7.09 ± 0.68**
4	Group IV – LC+ D-Galactosamine	200mg/kg + (400mg/kg)**	83.74 ± 2.19**	84.31 ± 1.95**	41.24 ± 1.37**	4.52 ± 1.27**	6.97 ± 1.53**
5	Group V – LC+ D-Galactosamine	400mg/kg + (400mg/kg)**	74.03 ± 1.73**	65.36 ± 1.21**	33.49 ± 1.04**	3.32 ± 1.63**	7.94 ± 1.32**

n=6; values were expressed Mean±S.E.M; ; Group II was compared to Group I. Groups III to V were compared to group II. \*p < 0.01 vs. D-Galactosamine group: significant; \*\* p < 0.001 vs. D-Galactosamine group: highly significant data were analyzed by One-way ANOVA followed by dunnett's 't' test.

**PCM- PARACETAMOL**

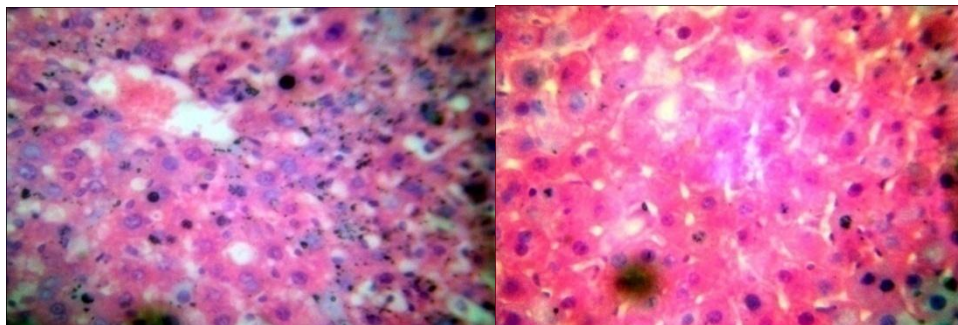
Histopathological examination of liver sections of control group (fig. 1) showed liver architecture is maintained. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval uniform nuclei. (fig. 2) showed liver with feathery degeneration and focal necrosis. The architecture was mildly distorted. The central veins were congested. There was patchy necrosis of the hepatocytes at focal areas. The portal

tracts showed mild chronic inflammation composed of lymphocytes. (fig. 3) showed liver. The architecture was normal. The central veins appeared normal. The hepatocytes showed round uniform nuclei and moderate cytoplasm. The portal triads showed mild peri-portal inflammation composed of lymphocytes. (fig. 4) showed liver with partially effaced architecture. The central veins are dilated and congested. The hepatocytes showed fatty steatosis. The portal triads showed periportal inflammation composed of lymphocytes. (fig. 5) showed liver. The architecture was mildly distorted. The portal triads are normal. The hepatocytes showed mild feathery degeneration. The portal triads appear normal.

### D-GALACTOSAMINE INDUCED

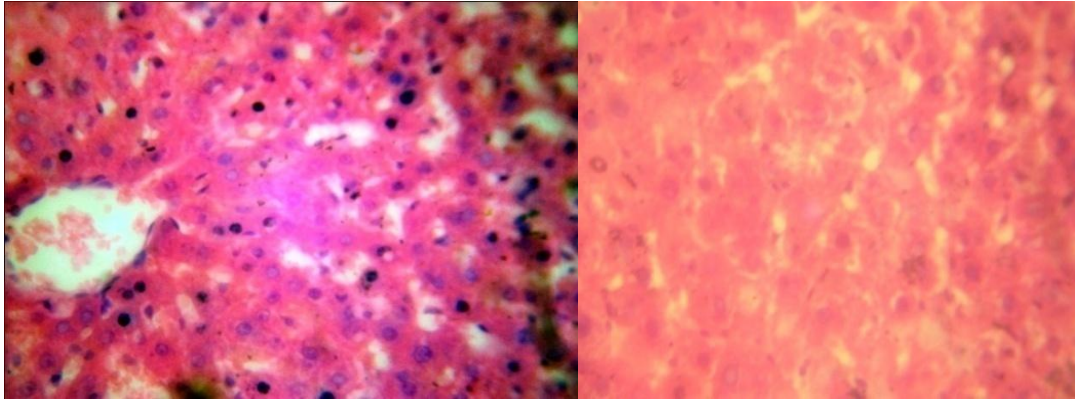
As shown in figure no 1 the architecture is maintained. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei. (fig. 2) showed liver with distorted architecture. The central veins were normal. There is patchy necrosis of the hepatocytes at focal areas. The portal tracts showed mild chronic inflammation composed of lymphocytes. (fig. 3) showed liver. The architecture was normal. The central veins showed mild congestion. The hepatocytes were normal and showed moderate cytoplasm and round uniform nuclei. The portal triads are normal. (fig. 4) showed liver with normal architecture. The central veins were congested. The hepatocytes show feathery degeneration. The portal triads show periportal inflammation composed of lymphocytes. (fig. 5) showed liver. The architecture was mildly distorted. The portal triads show inflammation. The hepatocytes appear normal and showed moderate cytoplasm and round to oval nuclei. There is no feathery degeneration of the hepatocytes.

### Histopathological Studies of liver (PCM induced)

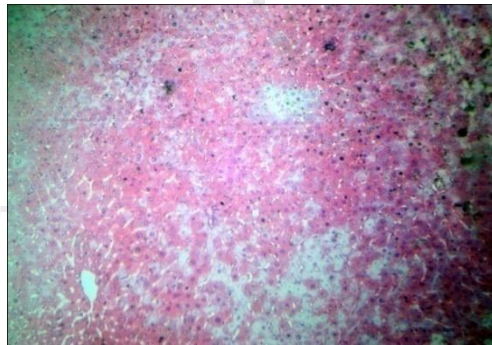


**Fig. 1: Histology of normal hepatic tissue** **fig. 2: PCM induced damage in hepatic tissue**



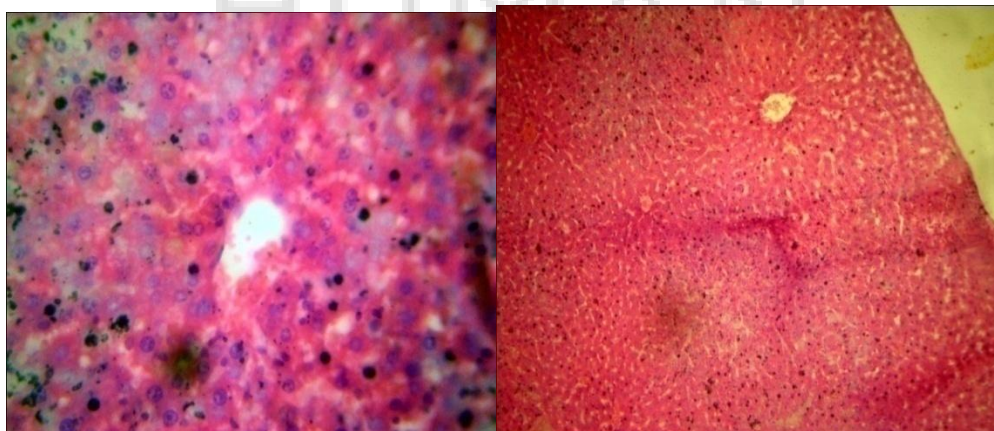


**Fig. 3:Effect of Silymarin on PCM induced hepatic damage Fig.4: Effect of LC (200mg) dose on PCM induced hepatic damage**

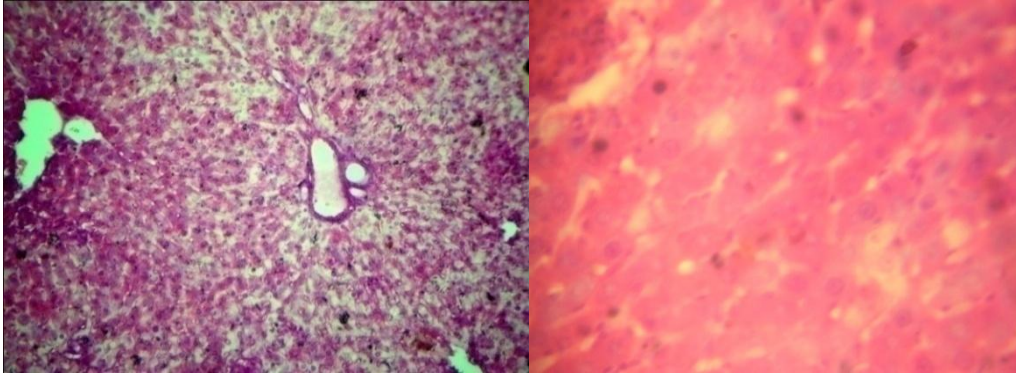


**Fig. 5:Effect of LC (400mg) dose on PCM induced hepatic damage**

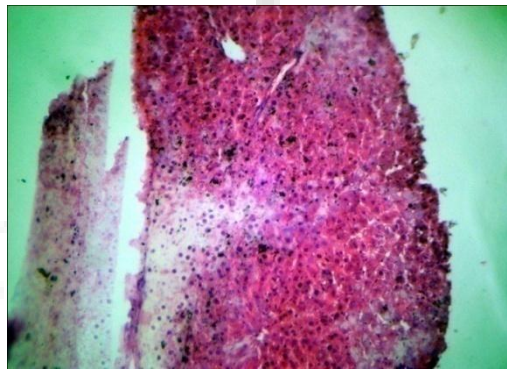
**Histopathological Studies of liver (D-Galactosamine induced)**



**Fig. 1: Histology of normal hepatic tissuefig. 2: D-Galactosamine induced damage in hepatic tissue**



**Fig. 3:Effect of Silymarin on D-Galactosamine induced hepatic damage Fig.4: Effect of LC (200mg) dose on D-GalactosamineInduced hepatic damage**



**Fig. 5:Effect of LC (400mg) dose on D-Galactosamine induced hepatic damage**

## DISCUSSION

The present study reveals the hepatoprotective activity of *Lindera communis* against Paracetamol and D-Galactosamine induced hepatic damage in rats. Hepatotoxic drugs such as D-galactosamine and acetaminophen reduces liver functional capacity, which leads to an accumulation of waste products such as ammonia in the blood (Mao et al., 2014). The results show that *Lindera communis* effective for hepatoprotection in low and medium doses (200 mg/kg, p.o and 400 mg/kg, p.o). Paracetamol is a commonly used as analgesic and antipyretic drug and is safe in therapeutic doses but produces fatal hepatic necrosis with toxic doses (Darbar S, and Bose A et al., 2009). The toxic effect of Paracetamol is due to oxidative damage induced by its metabolite, N-acetyl-p-benzoquinoneimine, produced by the action of cytochrome P-450 in the liver. This metabolite reacts with reduced glutathione (GSH) to yield non-toxic 3-GS-yl-

PCM. Depletion of GSH causes the remaining quinone and other natural endogenous oxygen species to bind to cellular macromolecules leading to cell death (Mitchell JR et al., 1973). D-Galactosamine is a well-established hepatotoxicant, which is widely used model which closely resembles human viral hepatitis in its morphologic and functional characteristics therefore considered very useful for evaluation of hepatoprotection (Udem SC et al., 1997; Langeswaran K et al., 2012). D-Galactosamine hepatotoxicity is considered as an experimental model of acute hepatitis although it does not affect other organs (Chaudhary CD et al., 2010). D-Galactosamine is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis. The toxicity of D-Galactosamine results from inhibition of RNA and protein synthesis in the liver. The metabolism of D-Galactosamine may deplete several uracil nucleotides including UDP-glucose, UDP-galactose and UTP, which trapped in the formation of uridine- diphosphogalactosamine. accumulation of UDP-sugar nucleotide may contribute to the change in the rough endoplasmic reticulum and to the disturbance of protein metabolism. Intense galactosamine of the membrane structures is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pumps, with consequent increase in the intracellular calcium is considered to be responsible for cell death (Jaishree V et al., 2010; Chaung SS 2003; Nakagiri, R et al., 2003).

This study shows that hepatic injury induced by Paracetamol and D-Galactosamine caused significant rise in marker enzymes SGOT, SGPT, ALP and total bilirubin. The serum enzymes like SGOT, SGPT, ALP and total bilirubin of treated animals were significantly reduced ( $p < 0.01$ ) by 28 days pretreatment of ethanolic extract of leaves of *Lindera communis* at two dose levels 200 mg/kg and 400 mg/kg p.o, when compared with Paracetamol and D-Galactosamine treated control (group II). From the result it is clear that the drugs show dose dependent activity. Histopathological observation also revealed that pretreatment with *Lindera communis* protected the animals from Paracetamol and D-Galactosamine induced liver damage. The results indicate that the leaves of *Lindera communis* possess the Hepatoprotective activity.

## CONCLUSION

From the present work we conclude that species of *L. Communis* are highly potential in biological activity. The preliminary screening of the samples revealed the presences of presences of high value of phenolic class of compounds as the major content in the plants.

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