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Hepato Protective Efficacy of *Terminalia chebula, Terminalia bellirica, Phyllanthus emblica* and Their Formulation on Imidacloprid Induced Liver Toxicity by Histopathological and Biochemical Parameters

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ABSTRACT

Terminalia chebula, Terminalia bellirica, Phyllanthus emblica and their formulations are used as a phytotherapeutic medicine for hepatic diseases. In the present study the effect of methanolic extract of herbal powder was administered against Imidacloprid in Albino Wistar Rats. Single dose of herbal (500 mg/kg/b.w) was given orally for 28 days to Imidacloprid toxicity exposed to rats. They were observed each hour and each day for 28 days for any changes in behavioural activity loss of ability to move, cramping, lethargy, muscle weakness, convulsion, irritation of the eye, excess salivation, change in gait. Clinical observations body weight, organ weight, biochemical analysis and histopathological examination were carried out. Administration of Imidacloprid, produced liver degeneration such as cytoplasmic vacuolation, mild vacuolar degeneration, mild hepatic damage in low dose (40 mg/kg b.w) and appearance of blood streaks, hepatic damage and severe vacuolar degenerations were observed in high dose of induction (80 mg/kg b.w). Animals were treated with the phytotherapeutic products of *Terminalia chebula, Terminalia bellirica, Phyllanthus emblica* and their formulations. Triphala extract was possessed more hepatoprotective activity, while comparing the four samples which was proved experimentally.

Keywords: Hepatoprotective, Phytotherapeutic, Imidacloprid (IMI), Histopathology, Methanolic.

INTRODUCTION

Human liver is the largest glandular organ in the body and weigh approximately 1.5 kg making about 2-3% of the total body weight. It is responsible for detoxifying the poisonous substances in the body by transforming and removing toxins and wastes. The most crucial is its role in the body's metabolism. There is no organ more important to healthy metabolism than the liver in many ways. [1] Liver occupies the pivotal position in body plays an essential role in drug and xenobiotic metabolism and maintaining the biological equilibrium of the organism. [2] Liver is exposed to absorb drugs and xenobiotics in the concentrated form and plays a central role in transforming and clearing the ingested drugs administered for therapeutic purposes or environmental xenobiotics. Hepatic metabolism is first and foremost mechanism that converts drugs and other compounds into products that are most easily excreted and usually have a lower pharmacologic activity than the potent compound. [3-4] A metabolic may have higher activity and /or greater toxicity than the original drug.

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Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction. [5]

A pesticide may be a chemical substance, biological agent (such as virus and bacterium), antimicrobial, disinfectant or device used against any pest. Insecticides can be classified according to the type of action into organochlorine, organophosphates, carbamates, pyrethroids, neonicotinoids, biological insecticides and antifeedants. [6] Imidacloprid is a neonicotinoid insecticide in the chloronicotinyl nitroguanidine chemical family. [7] Imidacloprid is a systemic insecticide was the first chloronicotinyl insecticide and is the largest selling insecticide worldwide. [8] Salivation and vomiting have been reported following oral exposure. Very high oral exposures may lead to lethargy, vomiting, diarrhoea, salivation, muscle weakness and ataxia, which are all indicative of imidacloprid's action on nicotinic receptors. Other signs of exposure at high doses are uncoordinated gait, tremors, and reduced activity. [9]

Triphala literally translated as 'Long Life Practice'. The three fruits contained in Triphala are Amalaki (*Phyllanthus emblica*), Haritaki (*Terminalia chebula*) and Bibhitaki (*Terminalia bellirica*). Amla comes from the fruit of a tree that is native to India, and it is a rich source of vitamin C. Harada is known as "the Tibetan king of medicine," and it act as famous tonic for the heart, brain as well as for long life, which is having the phytoconstituents, alkaloids, flavanoids, carbohydrates, saponins, tannin and polyphenols. [10] Baheda comes from a bitter fruit; it contains plenty of vitamin A, protein and omega 3-fatty acid. Bellirica and Emblica has OH-alcohol, CH-alkane, CF-alkyl halide, =CH-alkene and other functional groups reported in the previous study. [11] A popular saying in India goes, "No mother? No need to worry if you have triphala." They say this because they believe that triphala powder cleanses the organs just as a mother bathes her child. The present study aimed to study the role of methanolic extract of indigenous herbals (fruit) against Imidacloprid induced liver toxicity in wistar rats.

MATERIALS AND METHODS

Sample Collection and authentication

Fresh fruits of *Terminalia chebula Retz*., *Terminalia bellirica Roxb.* and *Phyllanthus emblica L.* were collected from hill areas, Atthipattu (Thiruvannamalai), Therambattu (Vellore) and Sirumalai (Dindugal). The samples were identified and authenticated by Dr. John Britto, Rapinet Herbarium, St. Joseph's College, Trichy, Tamilnadu, India and given the Voucher Specimen No. VEA/001/2013, VEA/002/2013 and VEA/003/2003 respectively**.**

Extraction

500 g fruits of *Terminalia chebula*, *Terminalia bellirica and Phyllanthus emblica* were shade dried, pericarp and mesocarp of fruits were pulverized into fine powder individually and formulated in 1:1:1 ratio using a stainless steel blender. Extracts were prepared by using Soxhlet extractor and 95% Methanol were used as solvent, the residue was filtered and concentrated under reduced pressure by rotary evaporator. The final extracts were stored in closed containers until further analysis. [12-13] Methanolic extracts of flowers were subjected to preliminary phytochemical screening of various constituents. [14-15]

Drugs and Chemicals

Imidacloprid 70% (W/G) was procured from Mercury Agro Agency at Kumbakonam, which was manufactured and marketed by Bayer Crop Science Limitted, Mumbai. The diagnostic chemicals were obtained from Biomarketting, Thanjavur and followed standard operating procedures.

Experimental Animals

Female albino wistar rats were selected as experimental animals between 6-8 weeks weighing and 160-180 grams were procured from Central Animal Facility, SASTRA University, Thanjavur, Tamilnadu, India. The rats were housed in solid bottom polypropylene cages, three rats per cage. Autoclaved rice husk was used as the bedding material and it was changed once in 3 days. The animals were maintained in the animal house sustained temperature at 22 ± 2°C and humidity 30-70% with light/dark cycle for 12 hours. The food provided for the animals were standard diet containing pelleted food and water *ad libitum* (Nutrilab Rodent Feed, Bangalore, India). The experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC), SASTRA University (Approval Number: 302/SASTRA/IAEC/RPP dated 29.04.2014). All the hygienic practice was followed during the maintenance of animals according to the guiding principles in the use and care of animals. Induction and treatment were given by ball tipped stainless steel feeding needle orally.

Experimental Design

Experimental animals after the adaptation of 2 weeks, rats were randomly assorted and allocated into 11 groups of six rat (n=6) in each group. Group I: These animals were maintained on normal diet and served as control, Group II: Imidacloprid (disease control - 40 mg/kg/b.w), Group III: Imidacloprid (disease control – 80 mg/kg/b.w) Group IV: Imidacloprid 40 mg/kg/b.w + *Terminalia chebula* 500 mg/kg/b.w, Group V: Imidacloprid 80 mg/kg/b.w + *Terminalia chebula* 500 mg/kg/b.w, Group VI: Imidacloprid 40 mg/kg/b.w + *Terminalia bellirica* 500 mg/kg/b.w, Group VII: Imidacloprid 80 mg/kg/b.w + *Terminalia bellirica* 500 mg/kg/b.w Group VIII: Imidacloprid 40 mg/kg/b.w + *Phyllanthus emblica* 500 mg/kg/b.w, Group IX: Imidacloprid 80 mg/kg/b.w + *Phyllanthus emblica* 500 mg/kg/b.w, Group X: Imidacloprid 40 mg/kg/b.w + Triphala 500 mg/kg/b.w and Group XI: Imidacloprid 80 mg/kg/b.w + Triphala 500 mg/kg/b.w orally for 4

weeks. Before necropsy blood was collected by retro orbital puncture in plain and heparinized tubes, centrifuged at 3000rpm for 10 minutes and the serum was separated for haematological and biochemical parameters, after sacrifice liver was removed and washed with saline, stored at 4°C for further antioxidant and histopathological investigations.

Histopathological Studies

For histopathological study the fresh liver tissues were collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100% v/v) cleared in Xylene by tissue processor (Leica TP 1020, made in Jerman) and embedded in paraffin. The paraffin embedding technique (Leica EG 1150C) was carried out; sections were done with microtome (Leica RM2125 RTS) at 5µm thickness. Sections were prepared and then stained with hematoxylin eosin dye by automatic staining (Leica ST 4040) for photographic microscopical studies were observed by Trinocular microscope (Nikon, Digital Sight DS-Fi2, Made in Japan).

Statistical Analysis

The data were statistically analysed and all values were expressed as mean ±SD. The data were also calculated by Duncan's Multiple Range Test (DMRT) $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Hepatoprotective activity was determined on the basis of microscopic examination of liver in Group II revealed cytoplasmic vacuolation, mild vacuolar degeneration and mild hepatic damage shown in figure 1B. Group III exhibited the appearance of blood streaks, hepatic damage and severe vacuolar degeneration depicted in figure 1C. Group IV, V, VI, VII, X & XI treated with herbal drugs were regenerated and the cells are exposed as normal hepatocytes displayed in figures 1D, 1E, 1F, 1G, 1J & 1K. Group VIII and IX treated with *Phyllanthus emblica* having bile cananiculus, which may be able to secrete and synthesize bile acids, pigments & bile salts. Metabolites of Imidacloprid are found in the liver and kidneys of rats after a single dose. Toxic signs like salivation, vomiting, lethargy, diarrhoea, muscle weakness and ataxia are indicative of Imidacloprid action on nicotinic receptors. [16] Liver and kidneys of male rats exposed to the sinusoids, hyperplasia of kuffer cells, mono nuclear cell infiltration and hydropic degeneration in liver at low doses (300 mg/kg) and congestion, mononuclear infiltration, tubular degeneration and fibrosis in kidneys at higher doses (600 mg/kg). [17]

The administration of Imidacloprid to the animals resulted in a significant rise in serum biochemical parameters Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Lactate Dehydrogenase (LDH) and declined in total protein, albumin, globulin, bilirubin, phosphorous and calcium shown the degeneration and regeneration of liver which is compared with Table 1 normal control. While categorize these parameters and comparing the therapeutic herbals triphala 40 mg/kg bw shown notable effect. *Phyllanthus emblica* extract has many pharmacological activities for the treatment of number of diseases and is a constituent of many hepatoprotective formulations. [18] In sub chronic oral toxicity study, wistar rats were treated with Imidacloprid at concentrations of 150, 600 and 2400ppm for a period of 13 weeks. Liver showed hypertrophy of hepatocytes and sporadic cell necrosis. The biochemical changes included elevated levels of Alanine Amino Transferase, Serum Alkaline Phosphatase with slight increase in blood clotting time. [19] The liver damage caused by pathogens as well as chemical agents are of similar nature and a proper treatment regime or a plan is absent for both. The lack of reliable liver protective drugs in allopathic medicine is explicitly inadequate. [20] Supportive treatment with vitamin supplements and anti-inflammatory drugs like colchicines only help to prolong the life span is a very limited manner. [21] Phenols, flavanoids, tannins and triterphenes are good antioxidants play a key role as hepatoprotective agents as reported by earlier studies. [22]

Hepatocytes are metabolic super-achievers in the body and play critical roles in synthesizing molecules that are utilized elsewhere to support homeostasis, in converting molecules of one type into another and in regulatory energy balance. [23] It is also the central site for the biotransformation of xenobiotics and therefore is involved in the detoxifying mechanism of the body. Liver is responsible for clearing the chemical toxins in the blood and in this process it is exposed to high concentration of toxicants and toxic metabolites making it very susceptible to injury [24] infections, hepatic dysfunctions and toxins are the major causes of hepatic injury. [25] Earlier study revealed that 1, 2, 3-Benzenetriol, Furfural, n-Hexadecanoic acid, 2-Furan Carboxaldehyde, 5-(hydroxy methyl)- and many other compounds were present are responsible for hepatoprotective effect. [26-27] Oral administration of Imidacloprid at 0.21 mg/kg b.wt for 28 days in male albino rats resulted in elevation of AST, ALT, ALP and MDA levels. [28] Oral administration of Imidacloprid at the rate of 80mg/kg b.wt for 28 days in male rats resulted in hepato toxicity which was evident from increased alanine transaminase and aspartate transaminase decrease in total protein and glutathione concentration in liver. [29] Liver, the largest organ in the body is essential in keeping the body functioning properly. It removes or neutralizes poisons from the blood, produces immune agents to control infection and removes microbes from the blood. It makes proteins that regulate blood clotting and produces bile to help absorb fats and fat soluble vitamins. Because of these activities it is exposed to a wide variety of insults and is therefore one of the most frequently injured organs of the body, yet one is unable to live without functioning liver. [25]

Table 1: Effect of IMI on biochemical serum parameters of experimental rats

Parameters & Groups	AST (U/L)	ALT (U/L)	Total Protein (gm/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Bilirubin (mg/dl)	Calcium (mg/dl)	Phosphorous (mg/dl)
Control	29.67± 1.21 ^{a}	15.67± 1.03 ^{a}	7.04±0.21 ^{a}	4.50±0.12 ^{a}	2.59±0.21 ^{a}	0.87±0.08 ^{a}	9.58±0.38 ^{a}	4.55±0.44 ^{a}
DC (40mg/kg)	52.00± 1.10 ^{b}	39.57± 0.50 ^{b}	4.55±0.06 ^{b}	3.14±0.07 ^{b}	1.31±0.17 ^{b}	1.68±0.04 ^{b}	5.57±0.21 ^{b}	1.98±0.14 ^{b}
DC (80mg/kg)	71.00±1.26 ^{c}	47.00±3.03 ^{c}	3.46±0.11 ^{c}	2.25±0.11 ^{c}	1.21±0.11 ^{b}	1.84±0.44 ^{b}	4.75±0.27 ^{c}	1.55±0.17 ^{c}
DC-40 + *T. chebula*	30.33± 1.37 ^{d}	24.20± 0.40 ^{d}	4.93±0.10 ^{d}	4.04±0.13 ^{d}	2.63±0.28 ^{a}	1.09±0.07 ^{c}	7.75±0.27 ^{d}	2.38±0.14 ^{d}
DC-80 + *T. chebula*	25.83± 0.75 ^{e}	26.00± 0.89 ^{e}	5.66±0.13 ^{e}	3.30±0.09	1.60±0.05 ^{c}	1.29±0.05 ^{d}	8.83±0.26 ^{ef}	3.05±0.14 ^{e}
DC-40 + *T. bellirica*	25.83± 0.75 ^{e}	26.00± 0.89 ^{e}	5.66±0.13 ^{e}	3.30±0.09	1.60±0.05 ^{c}	1.29±0.05 ^{d}	8.83±0.26 ^{ef}	2.66±0.28 ^{d}
DC-80 + *T. bellirica*	26.83± 0.75 $^{a\,e}$	30.17± 0.98 ^{f}	6.46±0.11 ^{f}	3.46±0.07 ^{e}	1.63±0.07 ^{c}	1.31±0.04 ^{d}	9.17±0.41 $^{a\,f}$	3.17±0.17 ^{e}
DC-40 + *P. emblica*	32.50± 7.77 ^{d}	21.67± 0.41 ^{g}	6.56±0.11 ^{f}	4.04±0.07 ^{d}	2.65±0.12 ^{a}	0.93±0.03 ^{a}	7.75±0.27 ^{d}	2.88±0.26 ^{e}
DC-80 + *P. emblica*	24.58± 0.49 ^{e}	31.38± 0.48 ^{f}	5.11±0.46 ^{d}	3.50±0.06 ^{e}	1.75±0.07 ^{c}	1.04±0.02 ^{c}	8.67±0.52 ^{e}	3.51±0.27 ^{f}
DC-40 + Triphala	30.33± 1.21 ^{d}	17.08± 0.66 ^{a}	7.50±0.20 ^{g}	4.68±0.13 ^{a}	2.68±0.10 ^{a}	0.83±0.04 ^{a}	9.25±0.42 $^{a\,f}$	3.73±0.36 ^{f}
DC-80 + Triphala	23.80± 0.69 ^{e}	22.33± 1.63 ^{g}	6.75±0.06 ^{h}	4.14±0.15 ^{d}	2.39±0.11 ^{f}	1.02±0.05 ^{c}	9.25±0.27 $^{a\,f}$	3.72±0.28 ^{f}

Values are expressed as Mean ± SD for six rats. Mean values within the column followed by different letters (Superscript) are significantly ($P < 0.05$) different from each other and same letters are non significant compared by Duncan's multiple range test (DMRT).

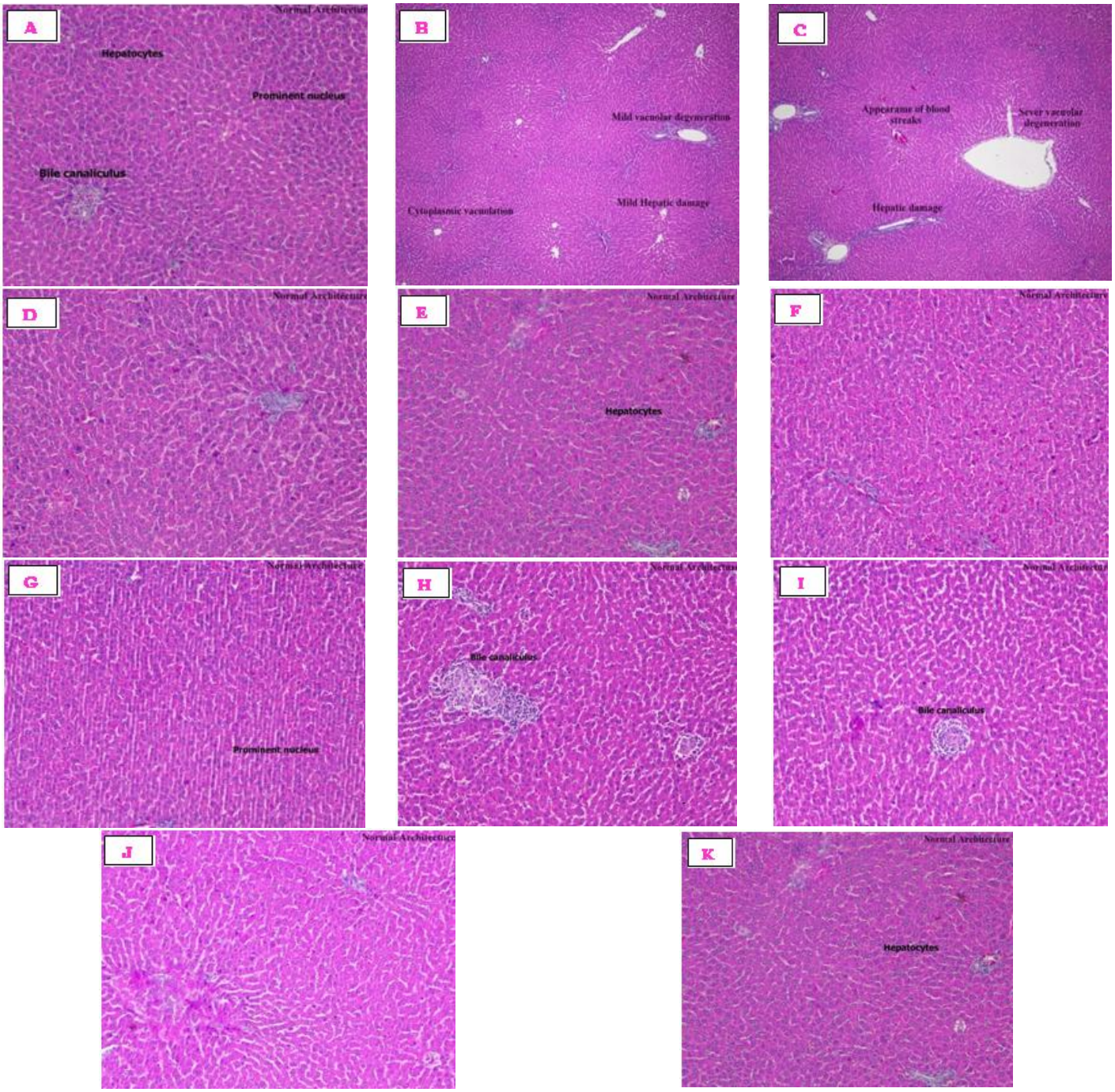

Fig. 1: Histopathological Examination of Liver tissue section in control and Experimental rats (Hematoxylin & Eosin, 10X)

A. Control, B. IMI - Disease control (40 mg/kg), C. IMI - Disease control (80 mg/kg), D. IMI (40 mg/kg) + *T. chebula*, E. IMI (80 mg/kg) + *T. chebula*, F. IMI (40 mg/kg) + *T. bellirica*, G. IMI (80 mg/kg) + *T. bellirica*, H. IMI (40 mg/kg) + *T. emblica*, I. IMI (80 mg/kg) + *T. emblica*, J. IMI (40 mg/kg) + Triphala, K. IMI (80 mg/kg) + T. Triphala

This study bare that the exposure to Imidacloprid (40 mg/kg) revealed low toxicity in hepatic cells and (80 mg/kg) exhibited moderate hepato toxicity in albino wistar rats. In herbal drug treated groups were recovered remarkably in low dose. Hence this study proved Imidacloprid induced organ toxicity where that is degeneration and the selected herbals were manifested and exposed regeneration of hepatocytes. Lack of allopathic medicines for the cure of hepatic injury exhorted the scientists to explore natural remedies and this may pave a way for the new phytotherapeutic inventions of the scientific world.

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