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Original Research

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Nephro-protective Effect of *Clerodendrum infortunatum* in Arsenic Induced Sub-acute Toxicity in Albino Rats

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Abstract

The nephro-protective effect of *Clerodendrum infortunatum* Linn (*C. infortunatum*) in arsenic induced sub-acute toxicity (28 days) was evaluated in albino rats. 18 albino rats were divided into 3 groups representing control, sodium arsenite alone and in combination with hydro-methanolic extract of *C. infortunatum* leaves representing Group I, Group II and Group III respectively. Sodium arsenite (3 mg/kg b.wt.) and *C. infortunatum* extracts (100 mg/kg b.wt.) were administered orally once daily over a period of 28 days. At the end of treatment period (29th day), rats were sacrificed. The blood samples for hematological parameters and kidney tissues for oxidative stress indices were collected. The kidney weight was also measured. The preliminary phytochemical screening of the above plant was done to know the different phyto-constituents present. Significant reduction were observed in kidney wt., TEC, Hb% and SOD levels where as creatinine, BUN, TLC and MDA levels ($P < 0.001$) were significantly increased on arsenic exposure. Treatment with extract of *C. infortunatum* @ 100 mg/kg b.wt for 28 days, significantly reduced the creatinine, BUN, TLC and MDA levels and significantly increased the kidney wt., TEC, Hb% and SOD level ($P \leq 0.001$) as compared to arsenic intoxicated group. The major phyto-constituents were present in the leaves i.e. alkaloids, flavonoids, saponins, sterols and terpenes. On the above findings, it can be concluded that treatment with *C. infortunatum* in sodium arsenite intoxication has significant role in protecting the animals from nephrotoxicity by modulating hemato-biochemical parameters and oxidative stress level.

Keywords: *Clerodendrum infortunatum*, Nephrotoxicity, Phytoconstituents, Rats, Sodium Arsenite, Sub-acute

1. Introduction

Arsenic is a widespread environmental contaminant distributed all around the world. It is associated with various health related problems including cardiovascular, hepatic, renal, immunological and reproductive system disorders. In addition to embryo toxicity and teratogenicity, tumors of skin, lung, liver, bladder and oxidative stress has also been reported on prolonged exposure of arsenic in men and animals. Contamination of water and soil by arsenic is a serious threat to mankind all over the world[1]. Arsenic occurs naturally in soil and minerals and enter the environment during the mining and smelting of ores, and get into water from runoff and leaching. Arsenic is also used in medicine and has various applications in agriculture, livestock and industrial sector. Arsenic enters the organisms through inhalation, dermal contact or ingestion of contaminated food and drinking water and redistributes itself to the entire organ system and accumulates and concentrates in the kidney during the urine elimination[2].

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Kidney is the major target organ for acute and chronic arsenic toxicity since they play a vital role in excretion[3]. Various epidemiological investigations have been observed showing dose-response relationship between arsenic in drinking water and several types of cancer[4]. Chronic exposure to arsenic causes damage to the kidney and increases the risk of renal cancer[4,5].

There is no such treatment for arsenic toxicity; researchers are now exploiting dietary antioxidants in the form of variety of plant products and their vital constituents have been utilized to combat arsenic induced toxicity. *Clerodendrum infortunatum* Linn, belongs to *Verbenacae* family and has been reported to have various pharmacological effects[6]. Since the biological activities were directly related to the bioactive constituents, investigations were carried out to examine the major phyto-constituents of aqueous-methanol extract of the *C. infortunatum* and its ameliorative and phytotherapeutic effect against arsenic induced nephrotoxicity in albino rats.

2. Materials and Methods

2.1 Extraction Procedure

Fresh leaves of plant were collected and identified by department of botany, RU, Ranchi. Leaves of the plant were subjected to solvent extraction. 250 gm shade dried powdered leaves of *C. infortunatum* was weighed and immersed in a hydro-alcoholic solution (20% distilled water + 80% methanol) in a flask stopper and was kept at room temperature for 48 hours and was stirred at interval at 150 rpm in an orbital shaker. The average time for extraction was 48 h. The extract was filtered, concentrated in vacuum followed by moist drying in hot water bath at 40–50^0 C. After scratching, dried powder of the extract was obtained[7]. The extractive value of methanolic extract was 17.67%. The testing sample was reconstituted by dissolving the solid mass with double distilled water for the oral administration in rats.

2.2 Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant under study was carried out in extract, using the standard procedures[8–10]. The tests were aimed to identify the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, sterols, terpenes, tannins and proteins.

2.3 Experimental Animals

The study was conducted on 18 adult healthy albino rats weighing 150–200 gms. Animals of either sex were randomly divided into three groups, containing six rats in each. The experimental animals were maintained in experimental animal laboratory, PG Department of Zoology, Ranchi University, Ranchi with good hygienic conditions in a clean propylene cages containing wheat straw chips as bedding with 12 h of dark and light cycle. The experimental rats were given standard feed and water ad libitum. Before start of experiment, animals were allowed for acclimatization for a period of seven days under constant observation.

2.4 Drug Used

Gr-1 Control – Normal saline for 28 days.

Gr-II – Sodium arsenite @ 3 mg/kg b.wt. single oral dose for 28 days.

Gr-III - The extract of *C. infortunatum* leaves @ 100 mg/kg b.wt along with Sodium arsenite @ 3 mg/kg b.wt. per day for 28 day orally.

2.5 Parameter Recorded

2.5.1 Organ Weight

All the rats were sacrificed at the end of the treatment period of 28 days. Kidneys were collected and weighted and were kept at –80^{0}C for the study of oxidative stress parameters. Blood was collected in heparinized/EDTA vials for hematological studies.

2.5.2 Hematological Parameters

The blood samples were collected by using EDTA (Ethylene Diamine Tetra Acetic Acid) as an anticoagulant and following parameters were estimated as per method described[11].

- Total Erythrocyte Count (TEC).
- Total Leucocyte Count (TLC).
- Haemoglobin percentage (Hb%).

2.6 Kidney Functioning Test

2.6.1 Creatinine Level

Creatinine was measured with the help of autoanalyser by the well established method[12]. Picric acid in alkaline medium reacts with creatinine to form an orange coloured complex with an alkaline picrate. The intensity of colour formed during fixed time was directly proportional to the amount of creatnine level and it is expressed in mg/dl.

Creatinine + Alkaline picrate ⟶ Orange colored complex

2.6.2 Blood Urea Nitrogen (BUN)

BUN was measured by the GLDH-urease method[13]. Urea reacts with water and releases ammonia (NH_3) and CO_2 in the presence of urease. The released ammonia reacts with NADH in the presence of Glutamate Dehydrogenase (GLDH) to form glutamate. The rate of oxidation of NADH to NAD is measured as the rate of decrease in absorbance is directly proportional to the amount of urea concentration present in the sample. BUN level is expressed in mg/dl.

2.6.3 Oxidative Stress Related Parameters

Frozen tissue samples were partially thawed and 1 g of tissue sample was weighed and 10% homogenate was prepared in ice-cold PBS with homogenizer (REMI) for about 1.5 min. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was used for the estimation of following oxidative stress indices.

- Lipid peroxidation (LPO) in tissue homogenate was estimated in terms of malondialdehyde (MDA) production by the modified method[14,15]. Calculation was done using the molar extinction coefficient of MDA-TBA complex at 532 nm i.e. 1.56×10^{-5}/mol/cm. The amount of lipid peroxidation is expressed in nano moles of MDA formed per mg of protein.
- Superoxide Dismutase (SOD) was estimated as per the method described by Madesh and Balasubramanium[16]. It involves generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide dependent reduction of the tetrazollium dye MTT to its formazan; the absorbance was measured at 570 nm in a spectrophotometer. The reaction was terminated by addition of Dimethyl Sulfoxide (DMSO), which helps to solublize the formazan formed. The enzyme activity is expressed as µM MTT formazan formed/min/mg protein, using a molar extinction coefficient of 17000 M^{-1} cm^{-1}.

2.7 Statistical Analysis

All experimental data were expressed as mean ± standard error of mean (SEM). Results are expressed as Mean ± SEM within equal to number of animals. Data were analyzed applying one way ANOVA using the Graph pad Prism v4.03 software program (San DIEGO, CA, USA) and the differences were considered statistically significant at $^{*}P<0.05$ or lower[17].

3. Results

3.1 Phytochemical Screening

The extract of *C. infortunatum* leaves was screened for the presence of major phytoconstituents. The presence of alkaloids, glycosides, sterol, terpene were found in higher extent and flavonoids were moderately present. The saponin and tannin were found in trace amount where as protein was absent, in the methanolic extract of *C. infortunatum* leaves (Table 1).

Kidney weight

The mean kidney weights of Group II animals were found to be significantly ($P<0.001$) lower as compared to control group whereas in Group III, the kidney weight was significantly increased as compared to control (Table 2, Figure 1).

Kidney toxicity marker

There was significant increase ($P \leq 0.001$) in creatinine and BUN values in Group II as compared to control group. The values were significantly lower in the Group III as compared to Group II. (Table 3, Figure 2 A–B).

3.2 Hematological Parameters

The mean values of Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC) and Haemoglobin (Hb%) during sub-acute toxicity study have been presented in (Table 4, Figure 3 A–C). The significant ($P \leq 0.001$) decrease in TEC and Hb% was recorded in Group II as compared to control and significant increased TEC and Hb% in Group III

Table 1. Qualitative phytochemical screening of methanolic extract of *C. infortunatum* leaves for its phytoconstituents

Phytoconstituents	Reagent	Reaction	Inference
Alkaloids: Dragendroff's reagent	1 ml extract + 4–5 ml dil. hcl + Dragendroff's reagent	Orange precipitate	++++
Mayer's reagent	1 ml extract + 4–5 ml dil. hcl + Mayer' reagent	White or pale-Yellow precipitate	++++
Carbohydrates (Molisch test)	1 ml extract + Molisch reagent + 1 ml conc. H_2SO_4 along sides of tube	Reddish violet ring at the interface	+
Flavonoids (Lead acetate test)	1 ml extract + 10% Lead acetate sol.	Yellow ppt.	+++
Glycosides (Keller-Kilani test)	1 ml extract+few drops glacial acetic acid + 1 ml Ferric chloride sol. + 2 ml conc. H_2SO_4	Reddish brown ring & upper layer turns bluish green	++++
Saponin (Foam test)	1 ml extract + Shaken well with water	Persistent foam	++
Sterols (Salkwoski test)	1 ml extract + 1 ml chloroform + 2 ml conc. H_2SO_4	Reddish brown ring at the interface	++++
Terpene (Libermann Test)	1 ml extract + acetic anhydride + 2 ml conc. H_2SO_4 along sides of tube	Reddish brown ring at the interface	++++
Tannin	1 ml extract + 1% Ferric chloride sol.	Greenish to blue black color	+
Protein	1 ml extract + Ninhydrin reagent + heat for 2–3 mins.	Violet color	_

Highly positive (++++), moderately positive (+++), weakly positive (++), Traces (+), Absent/negative (––)

Table 2. Effect of *C. infortunatum* extract on kidney weight (gms) in arsenic induced sub-acute toxicity in rats

Kidney weights	Group-1	Group-II	Group-III
	1.38 ± 0.03[a]	1.14 ± 0.02 [b]	1.25 ± 0.01 [c]

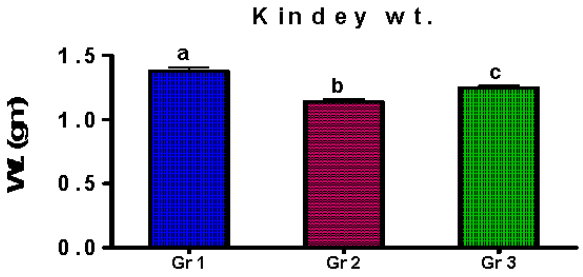


Figure 1. Histograms depicting the effects of *C. infortunatum* extract on kidney weight (gms).

as compared to Group II. There was a significant increase ($P \leq 0.001$) in TLC value in Group II as compared to control group. A significant decrease in TLC value was observed in Group III as compared to Group II.

Table 3. Effect of *C. infortunatum* extract on kidney function test i.e. creatinine (mg/dl) and BUN levels (mg/dl) (Mean±SE) in kidney tissue in arsenic induced sub-acute toxicity in rats

Groups	Creatinine (mg/dl)	BUN (mg/dl)
Group I	1.06 ± 0.041[a]	22.90 ± 0.78[a]
Group II	1.62 ± 0.12[b]	54.78 ± 1.42[b]
Group III	1.09 ± 0.04[a]	40.91 ± 1.17[c]

3.3 Oxidative Stress Parameters

The MDA and SOD levels in the kidney tissue of the groups are shown in Table 5, Figure 4 A–B. It was detected that a significant reduction was found in the activity of antioxidant enzyme SOD ($P < 0.001$) on arsenic exposure as compared to control, while the treatment of *C. infortunatum* significantly prevented impaired enzyme activity caused by arsenic. A significant increased in SOD value was observed in Group III as compared

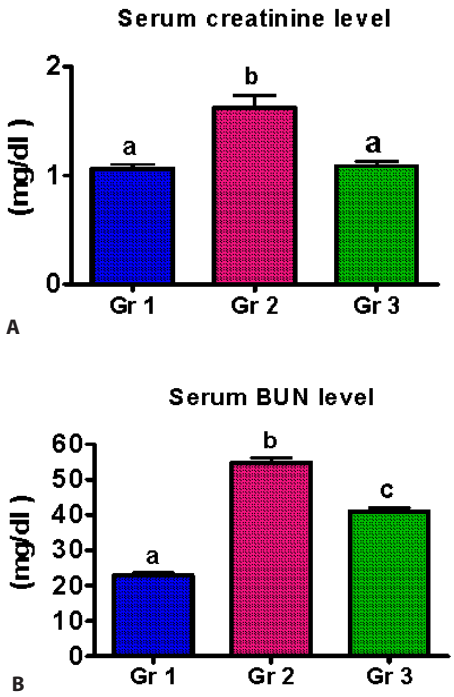


Figure 2. Histograms showing the effect of *C. infortunatum* on creatinine (mg/dl) and BUN (mg/dl) levels (Mean ± SE) in kidney tissue in arsenic induced sub-acute toxicity in rats.

Table 4. Effect of *C. infortunatum* on TEC (10^6/mm^3), TLC (10^3/mm^3), Hb (mg%) (Mean ± SE) in arsenic induced sub-acute toxicity in rats

Groups	TEC (10^6/mm^3)	TLC (10^3/mm^3)	Hb % (mg %)
Group I	8.26 ± 0.09[a]	9.62 ± 0.07[a]	15.12 ± 0.11[a]
Group II	6.63 ± 0.12[b]	12.62 ± 0.11[b]	12.10 ± 0.11[b]
Group III	7.52 ± 0.08[c]	10.57 ± 0.06[c]	14.05 ± 0.05[c]

Table 5. Effect of *C. infortunatum* extract on oxidative stress parameters, MDA level (nM. MDA produced/mg protein) and SOD level (U/g tissue) and (Mean±SE) in kidney tissue in arsenic induced sub-acute toxicity in rats

Groups	LPO (nM. MDA produced/ mg protein)	SOD (U/g of tissue)
Group I	43.15 ± 0.43[a]	8.34 ± 0.32[a]
Group II	52.83 ± 1.21[b]	4.90 ± 0.19[b]
Group III	47.63 ± 0.58[c]	6.25 ± 0.21[c]

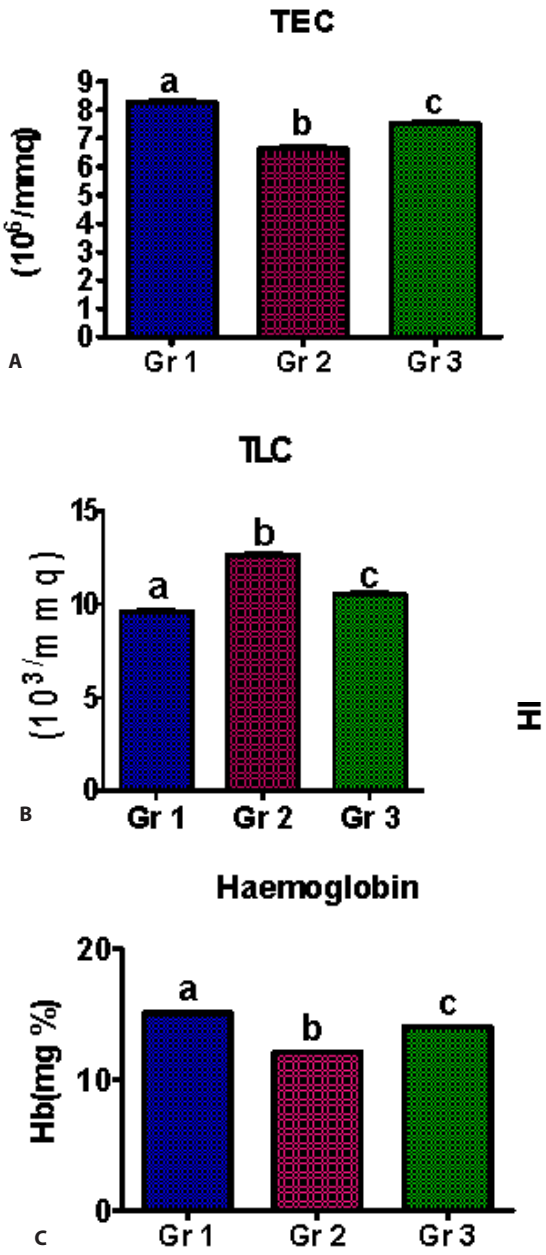


Figure 3. Histograms depicting the effects of *C. infortunatum* on (A) TEC (10^6/mm^3), (B) TLC(10^3/mm^3), (C) Hb (mg%) (Mean ± SE) in arsenic induced sub-acute toxicity in rats. (Different superscript representing significant values at 5% ($p < 0.05$).

to Group II (Table 5, Figure 4B). MDA level significantly decreased in the *C. infortunatum* treated group may consider to be associated with protective effect of *C. infortunatum* against oxidative damage caused by arsenic. (Figure 4A).

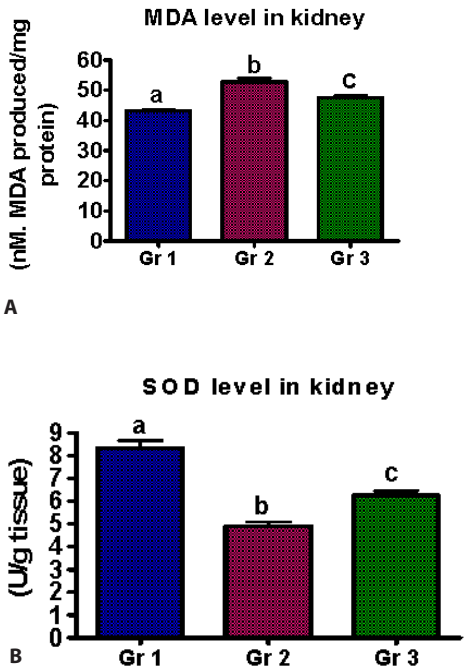


Figure 4. Histograms showing the effect of *C. infortunatum* on MDA (nM. MDA produced/mg protein) and SOD levels (U/g tissue) (Mean±SE) in kidney tissue in arsenic induced sub-acute toxicity in rats.

4. Discussion

Arsenic toxicity is a global health problem affecting many millions of people as well as animals. Contamination is caused by arsenic from natural geological sources leaching into aquifers, contaminating drinking water and may also occur from mining and other industrial processes. *C. infortunatum* is terrestrial shrub is widely distributed throutout India and also found in almost all districts of Jharkhand. The methanolic extract of *C. infortunatum* leaves contains alkaloids, glycosides, sterol, terpene in higher extent, flavonoids were found moderately and saponin and tannin were found in trace amount where as protein was absent. Various phyto-constituents may be responsible for wound healing activities, anti-inflammatory activities, antioxidant activities and antimicrobial activities[18,19]. Methanolic extract of *C. infortunatum* reported to contain polyphenolics and flavonoids that act as primary antioxidants or free radical scavengers[20].

Kidney is the main organ responsible for arsenic elimination and is considered as a target of arsenic toxicity due to its metabolites excretion[21]. Sub-acute toxicity of As decreased the weight and size of the kidney in rats of Group II as compared to Group I (Table 2). Kidney is vulnerable to arsenic-induced damage because of large perfusion and the increased concentration of excreted compounds that occurs in renal tubular cells[22]. Gupta et al., 2006 has also observed that renal damage in arsenic treated group may be due to oxidative stress induced by free radicals generation[23]. The kidney function markers like urea, creatinine etc. are breakdown products of normal body processes and are eliminated by the kidney. In the present study, the increased creatinine and BUN levels confirm the high degree of involvement of the renal tubular cells in the excretion of arsenic metabolites which make them more susceptible to damage and renal dysfunction. This results are also in accordance with other worker[24]. The presence of abnormally high levels of BUN and creatinine in serum are possible indicators of kidney injury induced through arsenic treatment. The serum creatinine level does not rise until at least half of the kidney nephrons are damaged or destroyed[25]. In the present study, significant increase in kidney weight was observed in sodium arsenite with *C. infortunatum* treatment. This was possible due to nephroprotective activity of *C. infortunatum* due to presence of antioxidants which was evident by observation of SOD and LPO in this study. Superoxide dismutase is an antioxidant enzyme that protect cell and tissue from the damage caused by free radicals and ROS by dismutating O_2^- into oxygen and H_2O_2, thus decreasing ROS generation and oxidative stress[26,27]. SOD of kidney was significantly decreased and increased LPO value in arsenic treated rats as compared to control group was observed. Increase in SOD and decrease in LPO were observed when sodium arsenite was administered with *C. infortunatum* which might be due to potent antioxidant activity of *C. infortunatum*. It might be due to presence of phenolics and flavanoids such as quercetin and kaempferol, having free radical scavenging activity in leaves of *C. infortunatum*[7].

The observation of heamatological parameters is very much important to correlate the kidney damage. There was reduced Total Erythrocyte Count (TEC) in sodium arsenite alone treated group (Table 3). The results obtained are in agreement with the results of previous studies[28,29]. This reduction in TEC might be due to inhibition of erythropoiesis by arsenic. The improvement of TEC in sodium arsenite plus *C. infortunatum* treated

group might be due to selective effect of the extract on erythroid compartment[5]. This might be due to cytoprotective and anti-oxidant present in *C. infortunatum* such as flavonoids, Vit E, Vit C and iron in the extract of leaves. There was also significant increase in the TLC values in arsenic treated group due to its intoxication and tissue necrosis. This might be due to participation of neutrophil in the process of phagocytosis and scavenges a wide variety of particulate material. The WBC count is regarded as non-specific predictor of various pathologic conditions including stress[30].

There was significant reduction in the Hb% in sodium arsenite treated group whereas improved Hb% in sodium arsenite and *C. infortunatum* treated group. This result is in agreement with previous work[29,31]. The reduction in Hb% in arsenic treated group may be due to inhibition of haemoglobin synthesis. About 85% of haeme-biosynthesis takes place in bone marrow and 15% in liver and other organs. The damage caused by arsenic in liver and bone marrow might be the reason of decreased Hb% in arsenic treated group. There may be haemolysis of red blood cells due to reaction of arsenic with oxygen. Arsenic binds with the sulphydryl group containing enzymes which are required for haemesynthesis, causing inhibition of these enzymes. The improved Hb% in group treated with sodium arsenite plus *C. infortunatum* might be due to the erythrocyte membrane integrity enhancing effect on red blood cells[32].

5. Conclusion

From the above obtained results, it can be concluded that As produces toxic effect on kidney and reduces body weight as well as organ weight in rats. The protective effects of *Clerodendrum infortunatum* against the sodium arsenite induced renal injury could be attributed due to its high levels of polyphenols and flavanoids antioxidants. It can be further exploited in other species to develop a prophylactic herbal product for the prevention of Arsenic intoxication.

6. Acknowledgement

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7. Conflict of Interest

There is no conflict of interest.

8. References

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