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ORIGINAL ARTICLE

Nutritional Value and Nephrotoxicity Effect of *Dillenia pentagyna* Fruit on Alloxan-induced Diabetic Rats

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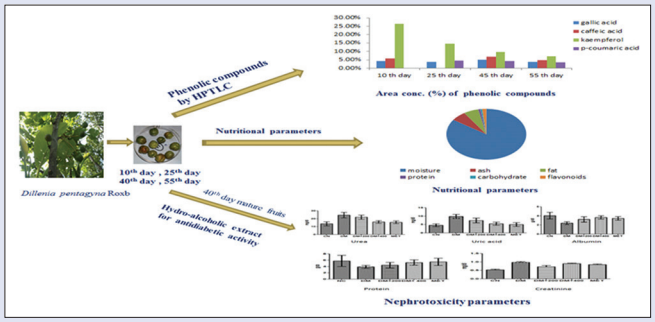
ABSTRACT

Background: *Dillenia pentagyna* Roxb. (*Dilleniaceae*) is an endangered medicinal plant, commonly known as "agai" and distributed randomly in the Terai belt of North East states of India. *D. Pentagyna* extract has been studied for therapeutic possessions. **Objective:** The proximate parameters and high-performance thin layer chromatography (HPTLC) analysis in the same plant of *D. pentagyna* of four-time harvest stage during the maturation of fruits were studied and also the antihypoglycemic activity of hydroalcoholic extract of *D. pentagyna* fruit. **Materials and Methods:** The proximate compositions were determined by measuring the amount of water removed from the food AOAC method and the chemical fingerprinting was carried out by HPTLC method. An acute oral toxicity study was carried out in healthy male Wister rats (110 g). The dose was finally made to 200 and 400 mg/kg body weight for oral administration after the LD_{50} estimation. **Results:** The most phenolic concentration was continuously decrease with during the maturation of fruits. After 21 days of treatment, level of blood glucose, as well as albumin, protein, creatinine, urea, and uric acid were significantly decreased when compared with the diabetic control. Hydroalcoholic (80% alcohol to 20% DW) extracts will be subjected to further extensive studies to isolate and identify their active constituents which are useful for against antidiabetic. **Conclusion:** *D. pentagyna* fruit could act as a source of functional compounds for the control of diabetes mellitus.

Key words: Diabetic nephropathy, *Dillenia pentagyna,* high-performance thin layer chromatography, hyperglycemic, medicinal plants

SUMMARY

- The hydroalcoholic (20:80) extract of *Dillenia pentagyna* fruit was antihyperglycemic activity analyzed
- Determine the nephrotoxicity parameters, i.e. albumin, protein, creatinine, urea, and uric acid were significantly decreased when compared with the diabetic control
- Four phenolic compounds were isolated by high-performance thin layer chromatography in the same plant of *Dillenia pentagyna* fruit during four-time maturity stage
- Six nutritional parameters were analyzed from the fresh fruit extract during four-time maturity stage of *Dillenia pentagyna* fruit.



Abbreviations Used: CN: Normal; AIDR: Alloxan-induced diabetic rat; DM: Diabetes mellitus; DN: Diabetic nephropathy; FGC: Final glucose concentration.

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INTRODUCTION

Medicinal plants are the record exclusive source of life-saving drugs and foods for the majority of the World's population. Diabetes mellitus (DM) is a health problem in worldwide, that takes a vital importance due to its high incidence.[1,2] *Dillenia pentagyna* Roxb. is a medicinal plant which fruits are used as vegetable and sourness.[3,4] The consumption of these plant fruit is widely regarded as beneficial for health, i.e., help in lowering incidence of degenerative diseases such as cancer, diabetes, arteriosclerosis, inflammation, brain dysfunction, and acceleration of the aging process.[5,6] Diabetic nephropathy (DN) is one of the important microvascular complications of DM. Hyperglycemia, the main determinant of the initiation and progression of DN not only generates more reactive oxygen metabolites but also attenuates antioxidative mechanisms through nonenzymatic glycosylation of anti-oxidant enzymes.[7] DM obtained by a persevering and exalted blood glucose concentration, salient to tangles that can be equipment and prolonged season.[8] The mechanism of alloxan action has been intensively studied, predominantly *in vitro* and is now characterized quite well. Using isolated islets and perfused rat pancreas, it was proved that alloxan denominates an emergent elevation in insulin ingestion in the presence or absence of glucose.[9] The emergent elevation in the blood insulin concentration was also fulfilled or comply with *in vivo* just after alloxan injection to rats.[10] The diabetogenic representative, alloxan, is having a tendency to mix with water and chemicals changeable compound. As an outcome, there is an urge to quest for

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compounds with effective antidiabetic activity when contracted orally.[11] Following a harvest period, fruits can have a relatively short shelf life during which they undergo profound changes in texture, color, and flavor.[12] The present study showed that biochemical changes affected the nutritional value of premature to mature period. Vitamin C (Ascorbic acid) is the most important vitamin in this fruit. The proximate parameters determine only the moisture content, total solid content, ash content, total fiber content, total fat content, carbohydrate content, ascorbic acid, and protein content.

This research focused on the analysis of the nutritional properties among four maturation stage, grown in the Terai region area, Bhinga forest region of Shravasti district, Uttar Pradesh, India. The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies.[13] The World Health Organization has projected that the number of diabetic patients will increase by the year 2025 with the current number 150 million to 300 million, which is carefully thought about as the silent widespread disease of 21st century. The Indian Council of Medical Research India Diabetes Study (ICMR-INDIAB) displayed that India had 62.4 million people with diabetes in 2011. These numbers are projected to increase to 101.2 million by 2030. ICMR-INDIAB homework exposed that the no of diabetic cases of type-2 DM, insulin-like growth factor, and impaired glucose tolerance (IGT) in north India are increased, in this region such as Chandigarh is which state among the selected region where the more incidence. The maximum incidence of type 2 DM, insulin-like growth factors and IGT in the north Indian center of Chandigarh.[14,15] However, we clearly knew that the use of oral anti-diabetics is limited due to their adverse side effects including hematological, hypoglycemic coma, and disturbances of liver and kidney functions.[16] In therapeutic practice, an impressive number of natural products have been presented for lead and model molecules for structure optimization and the expansion of more potent and/or better-tolerated remedies.[17] Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents.

MATERIALS AND METHODS

Plant material and sample collection

The fruits of *D. pentagyna* were collected from the Terai region area, Bhinga forest region of Shravasti district, Uttar Pradesh, India, and deposited in Ethno Botanical Herbarium, NBRI Lucknow, Uttar Pradesh, India. The specimen number of the plant is EBH No.: 265233. The fruits were harvested after different maturation stages at 10, 25, 40, and 55 days after full bloom. Each parameter was measured in three replications.

Sample preparation

The oven-dried fruit of *D. pentagyna* was subjected to pulverization to get a coarse powder. Hydroalcoholic (20:80) extract was made by Soxhlet methods for 15 h. The extracts were filtered through Whatman No. 1 filter paper and stored at 4°C used for further experiments. Acute oral toxicity study was carried out in healthy male Wister rats (110–150 g). The dose was finally made to 200 mg/kg and 400 mg/kg body weight (b.w.) for oral administration after the LD_{50} estimation. After the 21st days, animals were sacrificed, and blood was collected by the orbital sinus puncture method.[18] Blood was collected in a dried centrifuged tube and allowed to clot. Blood was centrifuged at 3000 rpm for 15 min at room temperature. The serum was collected carefully and kept at −20°C until analysis biochemical analysis. Biochemical parameters KFT (urea, uric acid, protein, albumin, and creatinine) were estimated according to the protocol of the manual of diagnostic kits.

Experimental design for animal study and ethical issues

Animals were kept under standard laboratory conditions (25°C ± 30°C, 12 h light/dark cycle) and had free access to food and clean tap water *ad libitum* for 28 days of the experimental period. The animal experiments were maintained in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines. All the procedures were in accordance with the Institutional Animal Ethics Committee Guidelines (UIP/IAEC/APRIL-2015/08). The experimental study was conducted on five groups of animals each with six Wistar albino rats were randomly allocated to each of the five groups.

The groups were treated as follows:

- Group I: (CN) - consisted of normal rats, orally given water and food
- Group II: (DM) - consisted of diabetic rats were received alloxan (150 mg/kg b.w.) by intraperitonially injection
- Group III: (DM + DP200) consisted of diabetic rats orally given *D. pentagyna* fruit extract by gavages (200 mg/kg b. w.) once daily for 28 days
- Group IV: (DM + DP400) consisted of diabetic rats orally given *D. pentagyna* fruit extract by gavages (400 mg/kg b. w.) once daily for 28 days
- Group V: (DM + MET200) consisted of diabetic rats orally given standard drug metformin (200 mg/kg b. w.) once daily for 28 days.

Proximate analysis

The samples were analyzed for proximate compositions which include moisture content, fat, ash, protein, fiber, flavonoids, carbohydrates, and sugar contents. The moisture and dry weight content in the food items were determined by measuring the amount of water removed from the food. It was done by direct heating the food in an Air oven at 100°C–105°C to constant weight. Ash in the food samples was estimated by heating the dried sample in a muffle furnace at 600°C for 3 h by AOAC method.[19]

Determination of crude fiber content

Crude fiber was estimated by gravimetric method as described by Raghuramulu method.[20] Extract 2 g ground material with petroleum ether to remove fat (40°C–50°C), after extraction with ether boil 2 g of dry material with 200 mL of sulfuric acid for 30 min and again filter through muslin and wash with boiling water then boil with 200 mL NaOH solution for 30 min. Filter through muslin paper and wash with 25 mL of boiling 1.25% H_2SO_4 then dry the residue for 2 h at 130°C ± 2°C and cool the dish in a desiccator and weigh (W_2). Remove the residue and transfer to ashing dish (pre weighed dish W_1) and after that ignite for 30 min at 600°C ± 15°C, cool in a desiccator and reweight (W_3).

Determination of crude fat content

Crude fat was estimated by diethyl ether method as described by Sadasivam and Manickam.[21] 2 g dry fruit sample kept in a Soxhlet thimble, required volume of solvent (petroleum ether) boiling point have 40°C–60°C. After extraction, we evaporate the excess of ether from the solvent flask on a hot water bath at 90°C for 30 min. The extracts were collected and cool the flask in a desiccator and weighs. When the process was completed, the ether was distilled and collected in another container and the remaining crude fat was dried and weighed.

Determination of the total nitrogen content

The Kjeldahl method uses sulfuric acid (H_2SO_4), a variety of catalysts and salts to convert organically bound N in plant tissue to ammonium (NH_4) with its subsequent measurement. The Kjeldahl procedure is the official

Table 1: Description of the proximate content value of *Dillenia pentagyna* fruits derived from selected maturation stages. Each value in the table was obtained by calculating the average of three determinants (*n*=3) and data are presented as Mean±SD

Proximate parameters	10 days	25 days	40 days	55 days
Moisture content (%)	82.41±0.93	82.69±0.87	87.65±0.99	87.99±0.81
Total solid content (%)	17.58±0.75	17.30±0.85	12.35±0.89	12.01±0.48
Ash content (%)	6.57±0.67	6.98±0.38	7.23±0.43	8.56±0.57
Crude fat content (%)	5.98±0.51	5.72±0.42	4.59±0.83	4.14±0.59
Protein content (g/100 g Fw)	0.92±0.06	0.56±0.07	0.43±0.02	0.24±0.01
Flavonoid content (g/100 g dw)	1.62±0.08	0.79±0.07	1.18±0.05	0.53±0.01
Carbohydrate content (g/100 g dw)	0.73±0.06	0.91±0.07	0.46±0.05	0.35±0.04
Reducing sugar content (g/100 g dw)	0.80±0.04	0.93±0.02	1.16±0.02	1.59±0.07
Non-reducing sugar content (g/100 g dw)	1.18±0.13	2.17±0.17	2.75±0.09	2.95±0.12

Table 2: Effect of *D. pentagyna* on body weight and organ/kidney weight in alloxan induced diabetic rat. The value represented as means±S.D for six mice per group. *P*<0.001 as compare to normal group and **P*<0.001 as compare to diabetic group

Treatments	Body weight (gm)		Kidney weight (gm)
	Initial	Final	
CN	41.03±1.65	42.14±1.85	1.40±0.15
DM	39.20±1.55	38.85±1.56	0.96±0.09
DM+200	40.03±1.69	41.14±1.86	1.20±0.15
DM+400	40.43±1.65	41.31±1.86	0.85±0.15

method of the American Association of Official Cereal Chemists[22] and the Association of Official Analytical Chemists.[23] Total nitrogen content was determined by the following procedures we applied from indicator (Mixed Indicator) and solvents (4% H_3BO_3, 40% NaOH and 0.1N H_2SO_4).

Determination of the protein content

Protein was estimated by the method[24] using bovine serum albumin (BSA) as a standard protein, it is a globular protein of molecular weight 68,000. It contains aromatic amino acids such as tyrosine, tryptophan, and phenylalanine and peptide bonds. Leaves (100 mg) and 50 mg of roots of treated and control plants were crushed in 3 mL of 10% chilled trichloroacetic acid and centrifuged at 10,000 g for 10 min. The absorbance was recorded at 660 nm using BSA as a standard.

Determination of the total carbohydrate content

The total carbohydrate was estimated from the method of Hodge and Hofreiter method.[25] A volume of 100 mg of the fruit sample in the boiling tube and hydrolyze by keeping it in a boiling water bath for 3 h with 5 mL of 2.5N-Hydrogen chloride after that cool at room temperature and neutralize with sodium carbonate until the effervescence ceases. Again make up the volume to 100 mL and centrifuge after that collect the supernatant and take 0.5 and 1 mL aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the working standards "0" serves as blank and make up the volume to 1 mL in all the tubes including the sample tube by adding distil water and then add 4 mL of anthrone reagent. Heat for 8 min in a boiling water bath and after that cool rapidly and read the green to dark green color at 630 nm.

Analysis of phenolic compounds by high performance thin layer chromatography

The separation followed by qualitative and quantitative analysis of compounds was performed by using CAMAG high performance thin layer chromatography (HPTLC) system equipped with Linomat IV applicator (Camag, Switzerland), CAMAG Thin Layer Chromatography (TLC) Scanner-3. HPTLC was performed on precoated silica gel HPTLC plate's $60F_{254}$ (20 cm × 20 cm) plate of 0.20 mm layer thickness. Sample and standard bands of 6 mm wide were applied to the plate under a flow of N_2 gas, 11.3 mm apart, 10 mm from the bottom edge, starting 8 mm from the edge of the HPTLC plate with Linomat IV applicator. The linear ascending development was carried out in CAMAG twin trough chamber (20 cm × 20 cm) which was presaturated with 25-mL mobile phase benzene:methanol:acetic acid (11.2:2:1) for 30 min at room temperature (25°C ± 2°C). The length of chromatogram run was 8 cm and TLC plates were air dried in fuming hood with adequate ventilation before scanning. The quantitative evaluation of the plate was performed in absorption mode at 282 nm, using slit width 6 mm × 0.45 mm at data resolution 100 µm step-1 and scanning speed at 10 mms-1 with a computerized TLC Scanner-3, Quantification of compounds in fruit extract were performed by using pure gallic acid, p-coumaric acid, chlorogenic acid, caffeic acid, quercetin, and kaempferol as external standard.

Statistical analysis

Statistical analysis was carried out under a completely randomized design with three independent experiments. All the experiments were carried out in three replicate ± Standard deviation. The data were analyzed by two way analysis of variance to confirm the variability and validity of results and Duncan's multiple range test was performed to determine significant differences between treatments.

RESULTS AND DISCUSSION

Changes in proximate content

At the four maturation stage, fruit weight and moisture content were gradually increased during maturation reaching the maximum level on the 55th day. The moisture, ash, reducing, and non-reducing sugar content increased with increasing days of the fruit maturity. Ash and sugar content positively correlated with increasing the humidity. While on the 55th day, protein, carbohydrate, crude fat, and total solid content sharply decrease with the increasing days of maturity. Sugar is one of the biochemical components of fruit virtue and their kinds and amount directly impression fruit-palate components such as mellowness.[26] Reducing sugar and nonreducing sugar content was augmented during the maturation period. The high carbohydrate content was recorded highest 0.91 g/100 g dw on day 25th day and lowest 0.35 g/100 g dw on 55th day. Protein content was found highest 0.92 g/100 g Fw on the 10th day and lowest 0.24 g/100 g Fw on 55th day as presented in Table 1.

Body and organs weight

The body and organ/kidney weight of control and experimental were given in Table 1. The total b.w. decreased as well as organ/kidney weight during diabetes, when compared with control rat ($P < 0.001$). The oral administration of aqueous extract (200 mg/kg b.w and 400 mg/kg b.w) significantly improved as shown in Table 2.

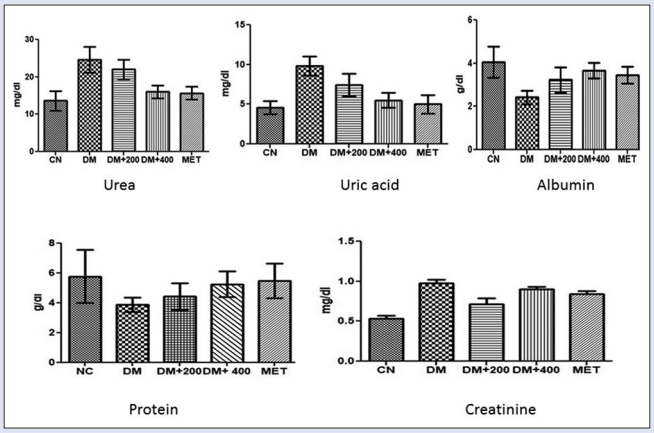

Figure 1: Effect of hydroalcoholic extract of diabetes mellitus +200 and diabetes mellitus +400 (200 and 400 mg/kg b.w.) on the serum urea levels, uric acid levels, albumin levels, protein and creatinine levels in alloxan-induced rat. Values are the means ± Standard deviation for six animals in each group. Values are significant at $P < 0.001$, statistical significance was compared within groups as follows. Diabetic rat was compared with normal rat. diabetes mellitus +200 and diabetes mellitus +400 treated diabetic rat were compared with diabetic mice (Diabetes mellitus)

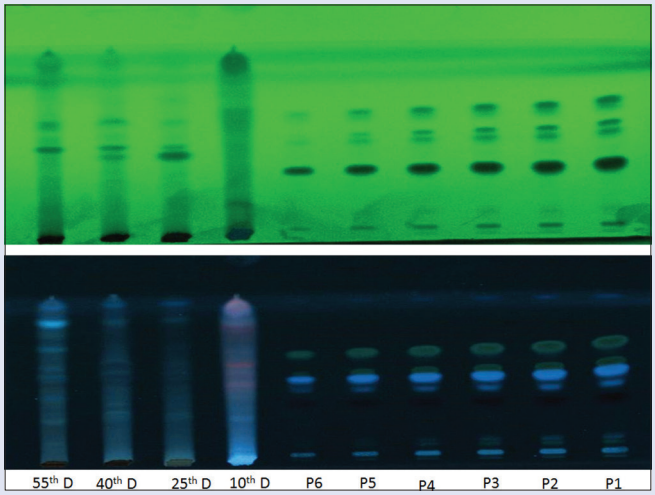

Figure 2: Chromatograms obtained from separation of plant extracts of 10th D, 25th D, 40th D, 55th D and standards P1: kaempferol; P2: p-coumaric acid; P3: chlorogenic acid; P4: caffeic acid; P5: quercetin; P6: gallic acid. Visualization was under ultraviolet light of wavelength 254 nm and 282 nm

Effect on serum glucose

Alloxan (150 mg/kg b.w.) administration resulted in significant elevation of glucose level. The administration of *D. pentagyna* at a dose of 200 and 400 mg/kg b.w. administered for 21st day were able to correct this aberration significantly ($P < 0.001$). The results of all the formulations tested are the following:

After induction of diabetes by alloxan, diabetes was confirmed by the presence of hyperglycemia in animals and the mean level of glucose in the control group of rat was evaluated to be 85.67 ± 12.03 mg/dl (range 60–95) whereas it was 298.5 ± 25.64 mg/dl (range 190–300, $P = 0.0001$) in alloxanized group. After the treatment of rat with the fruit extract of *D. pentagyna* (200 mg/kg b.w.), the glucose level decreased down to 234 ± 22.52 mg/dl ($P = 0.0001$) having a range of 156–245 mg/dl and more potent effect at the dose of 400 mg/kg b.w. of extract the level of glucose also significantly decreased to 134.3 ± 14.04 mg/dl ($P = 0.0003$) having range of 100–140 mg/dl. The significant increase in glucose concentration in the diabetic animals than that of the control mice is evident on alloxanization. However, the oral administration of aqueous extract of *D. pentagyna* significantly reduced the glucose level in serum when compared with alloxan-induced diabetic rat.

Kidney function level

The level of serum albumin, creatinine, protein, urea, and uric acid were presented in Figure 1. Significantly reduction in albumin and protein level ($P < 0.05$) was in alloxan-induced diabetic rats, compared to normal group and the level of urea, uric acid and creatinine were increased after alloxan induction result is similar to earlier reported.[25] On the other hand, oral administration of fruit extract of *D. pentagyna* on diabetic group, significantly decreased as well as the level of albumin and protein were increased.

Changes in high-performance thin layer chromatography fingerprint analysis

Four different mobile phases previously described for the separation of phenolics were tested, using silica gel HPTLC plates, namely benzene: methanol: acetic acid (11.2:2:1),[27] ethyl acetate:formic acid:acetic acid: water (100:11:11:26, v/v), ethyl acetate: methyl ethyl ketone: formic acid: water (50:30:10:10, v/v), and ethyl acetate:formic acid:water (82:9:9, v/v).[28] The only phase that allowed us to visualize differences among the extracts studied was the newly developed mobile phase benzene: methanol: acetic acid (11.2:2:1). The phenolic contents found in these plants showed the respective order as kaempferol > caffeic acid > p-coumaric acid > gallic acid. In 10th day, fruit sample was found only three phenolic compounds, i.e., gallic acid (4.35%), caffeic acid (5.81%) and kaempferol (26.46%). In 25th day, fruit sample was found only gallic acid (3.74%), p-coumaric acid (4.56%) and kaempferol (14.70%). In 40th day, fruit sample was found only four phenolic compounds such as gallic acid (5.17%), caffeic acid (6.91%) p-coumaric acid (4.33%), and kaempferol (9.65%). While, 55th day fruit sample was found four phenolic compounds such as gallic acid (3.89%), caffeic acid (4.77%) p-coumaric acid (3.45%), and kaempferol (7.02%). The kaempferol compound was continuously decreasing order with fruit development The florescence bands of most of the phenolics were visible at 254 nm wavelength as presented in Figure 2.

CONCLUSION

This study confirmed that the hydro-alcoholic extract of *D. pentagyna* (agai) fruit at 400 mg/kg b. w. dose exhibited significant antihyperglycemic than at low dose (200 mg/kg b.w.) in the induced diabetic rats. Agai fruit is a byproduct that could act as a source of practical amalgams in the treatment of DM and enhance the activity of synthetic oral hypoglycemic drugs. Natural foodstuffs are a good substitute with broader profits, within these few or valueless side effects. Our proximate result showed that *D. pentagyna* fruit have good source for nutraceuticals which possess various economic effects on human health. Our phenolics results showed that the most kempherol and gallic acid compound was decrease with the comparison of old stage (55th day). A major factor preventing the development of the medicinal plant-based industries in developing countries has been the lack of information on the social and economic benefits that could be derived from the industrial utilization of medicinal plants. As a result, determining the biological properties of plants used in traditional

medicine would be helpful to the rural communities and informal resolutions. Important information has been generated on antioxidants and nutritional properties of the samples of *D. pentagon* as they change with maturation time and system. These results showed that the concluded qualities generally change with time. Moreover, analyzing the nutritional and nephropathy factors and their levels in fruits can help in developing various processing methods that can lower their negative effects.

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Conflicts of interest

There are no conflicts of interest.

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