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Research Article

Antidiabetic Activity of Chloroform Extract of Carica papaya Cuticular Lipids (CECC) in Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycaemia. It is associated with various complications if not properly managed. This study was aimed to investigate the antidiabetic effects of chloroform extract of *Carica papaya* cuticular lipids (CECC) in alloxan-induced diabetic rats. In this study, CECC at 250, 500, 1000 and 2000 mg/kg body weight were administered to alloxan-induced diabetic rats for 21 days. Fasting blood glucose (FBG) level, lipid profile, liver and kidney function tests were conducted. All diabetic rats had initial FBG level ⩾200 mg/dL 72 hours after the induction. The extract was found to be safe up to the dose of 5000 mg/kg BW after acute toxicity studies. Oral administration of CECC significantly ($p< 0.05$) reduced FBG level, liver enzymes activities, serum potassium, bicarbonate and serum lipids levels (TC, TG, and LDL) in diabetic rats. Significant ($p<0.05$) increase in serum HDL was also noted in diabetic rats. This study concluded that the CECC have potential therapeutic effect in diabetes and related complications.

Keywords: *Diabetes mellitus, Carica papaya, Serum lipid profile, Blood glucose, Liver function test, Kidney function*

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder related to endocrine system (Li et al., 2004). It is a non-communicable disease and considered to be one of the five leading causes of death worldwide (Kandasamy et al., 2006). Its incidence is increasing rapidly in most parts of the world (Li et al., 2004). People suffering from diabetes lack the ability to synthesize or properly use insulin in the body which can lead to high level of glucose in the blood. Diabetes can also lead to elevation in plasma lipids which is a major risk factor for coronary heart diseases (Al-Hajj et al., 2021).

Diabetes mellitus causes high human, social and economic costs for countries of all income levels (Fagninou et al., 2019; Van Belle et al., 2011). The major signs and symptoms of diabetes mellitus include polyphagia, polyuria, polydipsia, weight loss, inability of body's healing capacity and blurring of vision (WHO, 2016).

Hyperglycaemia, which is the defined feature of uncontrolled diabetes, is associated with long term damage, dysfunction and eventually the failure of organs,

especially the eyes, kidneys, nerves, heart and blood vessels (WHO, 2016). Current strategies employed for the treatment of diabetes are targeted at ameliorating the different metabolic derangement associated with the disease in order to prevent or delay complications and maintain quality of life and through oral hypoglycaemic agents (Jugran et al., 2021; Davies et al., 2018).

Unfortunately, antidiabetic drugs are very expensive and have adverse effects including gastrointestinal disorders, hypoglycaemia, pancreatic degeneration, liver damage (Adjia Hamadjida et al., 2023). Medicinal plants have played a significant role in the palliation, management and cure of numerous diseases long ago. Natural products obtained from plants are essentially the great source of potential active ingredients (Cordell, 2000; S. Kumar et al., 2013).

Natural compounds isolated from various parts of the plant such as leaves, fruits, stem, roots, seeds have been shown to possess excellent medicinal value (Natarajan, 2014). Several medicinal plants have been studied for treatment of cancer, diabetes, arthritis, infectious diseases, etc., but there is still lack of scientific data for their efficacy in the treatment of such diseases. Carica papaya Linn. is one of such plant with potential medicinal value (Natarajan, 2014).

Carica papaya Linn (Caricaceae) is a type of tropical plant which is the only species in the genus Carica. It originated from the tropical regions of America and now widely cultivated in other tropical regions of the world (Vij & Prashar, 2015).

C. papaya effectively treats and improves all types of digestive and abdominal disorders. It is a medicine for dyspepsia, hyperacidity, dysentery and constipation. C. papaya helps in the digestion of proteins as it is a rich source of proteolytic enzymes (Aravind et al., 2013). Because of its high antioxidant contents, papaya can prevent cholesterol oxidation and can be used as a preventive treatment against atherosclerosis, strokes, heart attacks and diabetic heart disease (Ali et al., 2002).

In spite of these medicinal properties of C. papaya, its antidiabetic effect is yet to be investigated.

MATERIALS AND METHODS

Chemicals and reagents

Commercial kits for LFT, E/U/C, and lipid profile (assay kit from AGAPPE Diagnostics Switzerland GmbH) and purchased at Doyinson Nigeria limited, Alloxan monohydrate (Oxford Lab Fine Chem LLP). The standard antidiabetic drug Glibenclamide 5mg was produced from Hovid bhd, Malaysia and purchased at A.A Nasiha pharmaceutical limited Kofar guga area of Katsina State, Nigeria.

Fruit sample collection and identification

Carica papaya fruit waspurchased fromLambun Malam Lamis "Are" Dam, Rimi Local Government Area of Katsina state Nigeria in the month of November, 2023. The fruit was properly identified and authenticated in the Plant Science and Biotechnology unit of the Biology Department, Umaru Musa Yar'adua University, Katsina with a voucher specimen number UMYUH587.The fruit was subjected to extraction using chloroform.

Extraction of plant cuticular lipids

Fruit cuticular lipids of *Carica papaya* were extracted by completely dipping the fruit in two portions of 2 liter each of chloroform for 60 seconds and 30 seconds, respectively at room temperature. The extracts were then combined together and filtered using cellulose acetate filter with pore size of 0.45µm. Extracts were evaporated at 50°C to dryness, weighed and kept at -20°C until required for oral administration (Jetter *et al.,* 2018).

Experimental animals

Healthy adult albino rats (*Rattus norvegicus*) of both sexes between 2-3 months of age, weighing 150 – 200g were purchased from the Department of Microbiology, Bayero University, Kano. The rats were housed in a polycarbonate cage with unrestricted access to water and pellet meal (Vital feed®, Jos, Nigeria) at a temperature of 22°C. The animals were fully acclimatized for a period of two weeks (14 days) in the animal house of the Department of Biochemistry, Umaru Musa Yar'adua University, Katsina.

Acute toxicity studies

The median lethal dose (LD_{50}) of the cuticular wax extracts in Wistar Albino rats was determined using the method described by Lorke, (1983). Three groups of three rats each were orally administered with the extract at the doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight (BW) and observed for 24hours. In the second phase, four groups of three rats each were administered orally with the extract at doses of 1600mg/kg, 2900mg/kg and 5000mg/kg BW. The rats were observed for one week (7 days). Throughout the experiment, the changes in skin, eyes and fur were noted along with any sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and mortality.

Induction of diabetes in Wistar rats

Diabetes was induced in overnight fasted rats by intraperitoneal injection (IP) of alloxan monohydrate (150 mg/kg BW) freshly prepared in normal saline. To avoid alloxan-induced hypoglycaemia, due to the massive release of insulin from the pancreas, the rats were given 10% glucose solution in drinking water for 24 h after 6 hours of alloxan administration. The rats were monitored and examined for polydipsia, polyuria, polyphagia and general physical behaviors. The rats were starved for 24 hours, and onset of diabetes was confirmed using Accu-check glucometer (ACCU-CHECK Active, Mannheim, Germany) to measure their fasting blood glucose level 72 hours (3 days) after alloxan induction. Animals with blood glucose

level greater than 200 mg/dl (11.1mmol/L) were selected and allowed to stabilize for 3 days before the detailed experimentations (Asif *et al.,* 2019).

Experimental design and treatment of experimental rats

The animals were randomly allotted into eight groups consisting of five rats each. The groupings included: a Normal Control group; a Diabetic Control group that was induced with diabetes without treatment; a Standard Control group that was induced with diabetes and treated with 5 mg/kg BW of glibenclamide; Extract Control group, which was given 2000 mg/kg BW of extract of *Carica papaya* cuticular lipids (CECC); Diabetic groups that were induced with diabetes and treated with 250, 500, 1000 and 2000 mg/kg BW of CECC, respectively. Fasting blood glucose level and body weight was measured on weekly bases using a weighing balance (WTC 200, MRC-Laboratory equipment, UK).

Blood collection

At the end of 21 days of the experimental phase, all animals were fasted overnight. The animals were euthanized and blood samples were individually collected via cardiac puncture into plain bottles and allowed to clot, centrifuged at 200 rpm for 10 minutes, and the resultant sera were harvested for biochemical analysis.

Estimation of biochemical parameters

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), urea, creatinine, potassium, sodium, and bicarbonate were assessed using diagnostic kits (Randox laboratory, United Kingdom). The levels of triglyceride (TG), high-density lipoprotein cholesterol (HDL), and total cholesterol (TC) in serum were estimated using commercial kits lipid profile (assay kit from AGAPPE Diagnostics Switzerland GmbH). Low-density lipoprotein cholesterol (LDL) was estimated using the method of Friedewald *et al.* (1972).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using Statistical Package for Social Sciences (SPSS) software version 25. A p-value of less than 0.05 ($p < 0.05$) was considered to be statistically significant.

RESULTS

Acute toxicity studies

The CECC in Wister albino rats was found to be safe as the highest dose did not kill any of the experimental animals. Hence, the LD_{50} value was found to be > 5000 mg/kg BW and the extract is therefore safe for long term administration.

Body weight of experimental rats treated with *carica papaya* cuticular lipids extract

Bodyweight of the rats in diabetic control group decreased significantly ($p < 0.05$) after 21 days of the induction of diabetes as compared to the rats in the normal control group. However, treatment of the rats with different doses of CECC showed a significant ($p < 0.05$) gain in body weight compared to non-treated diabetic rats (Table 1).

Fasting blood glucose level of experimental rats treated with *carica papaya* cuticular lipids extract

The fasting blood glucose (FBG) level of the untreated diabetic group was significantly ($p<0.05$) elevated compared to normal control group on five days interval. Conversely, administration of different doses of CECC significantly ($p<0.05$) reduce the FBG level compared to untreated diabetic rats, except the diabetic group treated with 500 mg/kg BW where a significant increase was observed. Treatment of the non-diabetic rats (extract control group) with the CECC did not significantly ($p>0.05$) affect the FBG level compared to normal control group (Table 2).

Serum lipid profile of experimental rats treated with *carica papaya* cuticular lipids extract

The TC, TG and LDL- cholesterol levels of the untreated diabetic rats were significantly ($p<0.05$) elevated while the HDL-cholesterol concentration was significantly ($p<0.05$) lowered compared to the rats in the normal control group. While administration of different doses (250 and 500 mg/kg BW) of CECC significantly ($p<0.05$) reduce the levels of TC and LDL-cholesterol, the levels of TG and HDL-cholesterol significantly ($p<0.05$) increase compared to non-treated diabetic rats. Treatment of the non-diabetic rats (extract control group) with CECC did not significantly ($p>0.05$) affect the levels of serum lipids compared to normal control group (Table 3).

Liver function biomarkers of experimental rats treated with *carica papaya* cuticular lipids extract

The activities of serum AST, ALT, and ALP of the untreated diabetic group were significantly ($p<0.05$) elevated compared to the normal control group. There were significant ($p<0.05$) decrease in serum ALT and AST activities and a significant ($p<0.05$) increase in serum ALP activity after 21 days of rats treatment with 250, 500 and 1000 mg/kg BW of the extract compared to the diabetic control group. Treatment of the non-diabetic rats (extract control group) with the CECC did not significantly ($p>0.05$) affect the level of biochemical makers of hepatic function compared to normal control group (Table 4).

Renal function indices of experimental rats treated with *carica papaya* cuticular lipids extract

The levels of urea, sodium, potassium, creatinine and bicarbonate were significantly ($p<0.05$) elevated in the

non-treated diabetic rats compared to the rats in the normal control group. There were significant ($p<0.05$) decrease in the levels of potassium and bicarbonate and a significant ($p<0.05$) elevation of urea, sodium and creatinine concentrations following treatment of rats with different doses of CECC as compared to non-treated diabetic rats. Treatment of the non-diabetic rats (extract control rats) with the extract did not significantly ($p>0.05$) affect the level of biochemical makers of renal function compared to normal control group (Table 5).

Table 1: Effect of *Carica papaya* Cuticular Lipids Extract on Body Weight (g) of Diabetic Rats

Group	DAY 0	DAY 7	DAY 14	DAY 21
Normal Control	182.48 ± 19.85[a]	184.65 ± 19.63[a]	185.95 ± 21.09[a]	187.4 ± 19.77[a]
Diabetic Control	168.6 ± 18.58[b]	167.6 ± 18.11[b]	165.28 ± 17.93[b]	157.58 ± 18.92[b]
Standard Control	185.8 ± 20.66[a]	185 ± 20.38[a]	185.9 ± 20.31[a]	186.75 ± 21.90[a]
Extract Control	170.73 ± 7.52[a]	172.55 ± 6.22[a]	173.08 ± 7.08[a]	173.68 ± 6.93[a]
Diabetic + 250mg/kg BW of CECC	182.48 ± 15.94[a]	180.9 ± 15.92[a]	179 ± 15.11[a]	177.85 ± 14.90[a]
Diabetic + 500mg/kg BW of CECC	176.78 ± 22.32[a]	175.75 ± 21.49[a]	174.38 ± 21.39[a]	173.5 ± 21.67[a]
Diabetic + 1000mg/kg BW of CECC	182.48 ± 17.91[a]	181.78 ± 18.43[a]	180.08 ± 18.74[a]	178.83 ± 18.44[a]
Diabetic + 2000mg/kg BW of CECC	186.9 ± 11.92[a]	186.03 ± 11.92[a]	182.65 ± 9.42[a]	184.68 ± 11.87[a]

Data are expressed as mean ± SD. Values with different superscript alphabets down a column are significantly ($p < 0.05$) different from each other.

Table 2: Effect of *Carica papaya* Cuticular Lipids Extract on Fasting Blood Glucose (mg/dL) Level of Diabetic Rats

Group	DAY 0	DAY 5	DAY 1O	DAY 15	DAY 21
Normal Control	106 ± 3.84[a]	105.18 ± 3.32[a]	107.75 ± 7.50[a]	101.25 ± 2.77[a]	107 ± 5.15[a]
Diabetic control	225.5 ± 15.01[b]	225.25 ± 17.94[b]	229.75 ± 20.09[b]	222 ± 19.07[b]	232.25 ± 19.72[b]
Standard drug	242 ± 39.10[c]	240 ± 39.03c	226.5 ± 27.45c	193.75 ± 44.35c	188.75 ± 27.15c
Extract control	105.75 ± 5.45[a]	106.5 ± 7.43[a]	109 ± 5.24[a]	110 ± 2.12[a]	108 ± 5.34[a]
Treatment - 250MG/KG	221 ± 15.51[b]	220 ± 15.25[b]	227.5 ± 17.40[b]	234.5 ± 32.87[b]	254.5 ± 24.19[b]
Treatment - 500MG/KG	238.75 ± 36.08[b]	241.25 ± 35.61[b]	241 ± 35.73[b]	238.5 ± 43.29[b]	241.5 ± 33.69[b]
Treatment - 1000MG/KG	221 ± 16.39[c]	219 ± 17.59[c]	214.75 ± 14.02[c]	212.25 ± 9.58[c]	212.5 ± 11.61[c]
Treatment - 2000Mg/Kg	219 ± 6.75[d]	217.5 ± 3.35[d]	212.25 ± 7.98[d]	211 ± 7.84[d]	211.25 ± 6.87[d]

Data are expressed as mean ± SD. Values with different superscript alphabets down a column are significantly ($p < 0.05$) different from each other.

Table 3: Effect of *Carica papaya* cuticular lipids extract on serum lipid profile of diabetic rats

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal Control	41.03 ± 9.73[a]	4.38 ± 0.61[a]	5.93 ± 3.93[a]	36.35 ± 12.06[a]
Diabetic control	69.23 ± 7.20[b]	8.45 ± 2.26[b]	1.63 ± 0.96[b]	66.18 ± 10.67[b]
Standard drug	80.68 ± 14.63[c]	43.43 ± 20.14[c]	2.98 ± 2.07[c]	54.83 ± 12.76[c]
Extract control	30.23 ± 7.07[a]	5.95 ± 2.57[a]	5.93 ± 4.10[a]	43.8 ± 7.12[a]
Treatment - 250MG/KG	67.78 ± 10.47[b]	20.43 ± 5.68[b]	2.95 ± 1.69[b]	49.28 ± 16.22[b]
Treatment - 500MG/KG	68.13 ± 7.06[b]	21.45 ± 1.31[b]	5.33 ± 0.96[b]	59.23 ± 0.97[b]
Treatment - 1000MG/KG	101.23 ± 25.49[c]	22.45 ± 3.53[c]	4.45 ± 3.24[c]	82.03 ± 26.71[c]
Treatment - 2000Mg/Kg	95.43 ± 10.20[c]	18.75 ± 2.11[c]	7.28 ± 4.22[c]	75.15 ± 21.67[c]

Data are expressed as mean ± SD. Values with different superscript alphabets down a columnare significantly ($p < 0.05$) different from each other.
TC, Total cholesterol; TG, Triglyceride; HDL, High-density lipoprotein cholesterol; LDL, Low-density lipoprotein cholesterol.

Table 4: Effect of Cuticular Lipids Extract on Liver Function biomarkers of Diabetic Rats

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control	12.25 ± 5.12[a]	16 ± 18.32[a]	12.85 ± 6.15[a]
Diabetic control	15.5 ± 3.20[b]	73.5 ± 11.52[b]	18.2 ± 1.14[b]
Standard drug	14.75 ± 5.67[b]	22.75 ± 11.12[b]	23.73 ± 11.17[b]
Extract control	11.25 ± 2.95[a]	22 ± 10.46[a]	9.48 ± 4.38[a]
Treatment - 250MG/KG	10.25 ± 1.92[a]	35 ± 6.16[a]	18.4 ± 1.04[b]
Treatment - 500MG/KG	11 ± 1.22[a]	21.25 ± 4.66[a]	18.65 ± 1.69[b]
Treatment - 1000MG/KG	12.25 ± 5.97[b]	50.25 ± 32.87[b]	20.83 ± 3.60[b]
Treatment - 2000Mg/Kg	14.5 ± 4.77[b]	105.75 ± 56.97[b]	18.2 ± 1.14[b]

Data are expressed as mean ± SD. Values with different superscript alphabets down a column are significantly ($p < 0.05$) different from each other.
ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase.

Table 5: Effect of Cuticular LipidsExtract on Kidney Function Indices of Diabetic Rats

Group	Urea (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Creatinine (mEq/L)	Bicarbonate (mg/dL)
Normal Control	51.25 ± 7.4[a]	365.68 ± 76.7[a]	11.93 ± 2.7[a]	0.83 ± 0.37[a]	76.5 ± 8.41[a]
Diabetic control	52 ± 5.43[b]	372.5 ± 41.46[b]	20.13 ± 3.16[b]	1.05 ± 0.4[b]	98.5 ± 13.29[b]
Standard drug	51.75 ± 4.21[a]	385.83 ± 15.55[a]	18.98 ± 3.22[a]	1.15 ± 0.57[a]	80.25 ± 3.96[a]
Extract control	50.75 ± 7.53[a]	407.88 ± 43.06[a]	11.33 ± 3.74[a]	1 ± 0.67[a]	87.5 ± 13.38[a]
Treatment - 250MG/KG	52.25 ± 8.23[a]	381.93 ± 45.12[a]	19.73 ± 1.88[a]	1.35 ± 0.4[b]	92.25 ± 21[b]
Treatment - 500MG/KG	57 ± 12.63[b]	398.28 ± 57.12[b]	17.58 ± 2.05[b]	1.1 ± 0.14[b]	93.25 ± 6.83[b]
Treatment - 1000MG/KG	53.5 ± 7.4[b]	381.33 ± 57.47[b]	20.13 ± 3.33[b]	1.08 ± 0.23[b]	71.75 ± 4.66[b]
Treatment - 2000Mg/Kg	53.75 ± 11.12[b]	399.48 ± 60.74[b]	20.05 ± 1.32[b]	1.2 ± 0.56[b]	65.75 ± 5.26[b]

Data are expressed as mean ± SD. Values with different superscript alphabets down a column are significantly ($p < 0.05$) different from each other.

DISCUSSION

Diabetes mellitus, a chronic metabolic disorder characterised by elevated blood glucose level (hyperglycemia) (Banday et al., 2020). Uncontrolled diabetes mellitus can lead to various complications such as cardiovascular diseases, neuropathy, nephropathy, and retinopathy (Banday et al., 2020). Alloxan monohydrate induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (Cnop et al., 2005; Szkudelski, 2001). Alloxan monohydrate acts by generating reactive oxygen species that damage the pancreatic β-cells where insulin is produced, resulting in decreased levels of insulin. This process can lead to diabetes (Milagro & Martínez, 2000). In this study, increase in blood glucose level was observed in alloxan-induced diabetic rats. This observation correlates with the previous research finding in that the blood glucose level significantly increased in alloxan-induced diabetic rats (Verma et al., 2010). Polyphagia, polydipsia, polyuria and concomitant reduction in body weight which are classical symptoms and signs of diabetes were also observed in diabetic rats in this study (Asif et al., 2019; Sridhar et al., 2005).

Depletion of body weight was observed in diabetic rats. This is supported by the findings of Asif et al. (2019), Dheer & Bhatnagar, (2010) and Abu et al. (2023). Significant increase in body weight was found in the diabetic rats treated with CECC. The body weight gain suggests that the extract has antidiabetic efficacy. Blood glucose elevation was observed in alloxan induced diabetic rats.

This observation correlates with the previous research findings (El-Demerdash etal., 2005; Subramaniam et al., 2011; Verma et al., 2010). The significant increase in the level of blood glucose in the diabetic rats were significantly reduced upon treatment with the CECC for 21 days. It is worthy of note that the high doses of the extract causemore significant reduction in the blood glucose level. Hyperlipidaemia is one of the major complications of untreated diabetes. It occurs as a result of excess mobilization of fat from the adipocyte of adipose tissues due to under-utilization of glucose in diabetes (Nimenibo-Uadia, 2003).

In this study, induction of DM using alloxan significantly ($p < 0.05$) increased the concentrations of serum urea, creatinine, sodium, potassium and bicarbonate. This observation is supported by the experiment carried out by Abu et al. (2023). Treatment of the diabetic rats with the CECC reduces the concentrations of potassium and bicarbonate. This implies that the extract has therapeutic effect on renal diabetes-associated complications.

Elevation in the activities of liver enzymes (ALT, AST and ALP) was observed in diabetic rats. The increase in the activities of serum AST and ALT indicated that diabetes may have induced hepatic dysfunction, treatment of the diabetic rats with the extract lead to a significant decrease in serum biomarkers of hepatic function (AST, ALT and ALP). This implies the potential of the extract to ameliorate hepatic diabetes-associated complications.

CONCLUSION

The glycemic study found that the CECC has antidiabetic effect,which confirm the plausibility of traditional claims. Our future research will focus on finding the active compounds present in the CECC and their possible pharmacological mode of action.

AUTHORS' CONTRIBUTIONS

Conceptualization: NUM, AN, MMU; Methodology: NUM, A.N, MMU, UAI; Validation: NUM, AN, MB; Formal analysis and investigation: NUM, AN, MMU, IHK; Resources: MM, NUM, AN; Data curation: NUM AS, MMU; Writing - original draft preparation: MMU; Writing-review and editing: NUM, AN, MMU, MB, IHK, AIY, SY, AU, ASA, AMY: Supervision: NUM, AN, MB; Project administration: NUM, AN, MB; Funding acqusition: MMU, NUM. All authors contributed to this research have read the manuscript and gave final approval of the version to be published.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is an original research work

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