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Comparative study of antidiabetic and hypolipidaemic activity of leaf extract of Gymnema sylvestre with Glibenclamide in alloxan induced diabetic rabbits

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Abstract
Objectives: To evaluate the antidiabetic and Hypolipidaemic effect of Gymnema sylvestre in comparison with Glibenclamide in alloxan induced diabetic Rabbits.
Materials and method: Rabbits were randomly divided into 3 groups, each comprising 6 rabbits. Group 1 - Normal control distilled water, Group 2 - Test drug Gymnema sylvestre extract - 800mg/kg, Group 3 - Standard drug Glibenclamide - 0.5mg/kg was administered orally for 30 days. The fasting blood glucose and serum lipid profile were estimated in both normal and alloxan induced diabetic rabbits.
Results: The percentage reduction in FBS after 30days of treatment in Gymnema sylvestre group 69.91% and in Glibenclamide group 65.75%, difference between groups was statistically significant. P value - 0.0139**. The percentage reduction in lipid levels after 30days of treatment in Gymnema sylvestre group were TG-30%; TC-38%; HDL-12.30%;LDL-78.78% and in Glibenclamide group were TG-9.6%;TC-30%; HDL-4%; LDL-45%. There was statistically significant difference between groups in TG, TC, HDL except LDL. P value TG (0.002**), TC (0.02*), HDL (0.0004***), LDL (0.067).
Conclusion: The leaf extract of Gymnema sylvestre showed excellent antidiabetic potential and equally effective when compared with Glibenclamide in alloxan induced diabetic rabbits. Hypolipidemic effect of Gymnema sylvestre should be further evaluated in comparison with standard hypolipidemic drugs.

Keywords: Diabetes, Hyperlipidemia, Gymnema sylvestre, Glibenclamide.

Introduction
Diabetes mellitus is the commonest metabolic disorder characterized by chronic hyperglycemia and derangement of carbohydrate, fat and protein metabolism due to absolute or relative deficiency of insulin or its action.
Long standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body particularly the vascular system. This leads to complications like diabetic neuropathy, nephropathy, retinopathy, etc. Glycemic control prevents the development of long term complications of diabetes mellitus [1].
Dyslipidemia is a relatively common problem in patients with poorly controlled diabetes mellitus. It has been estimated that the frequency of elevated plasma lipid levels in diabetic patients is between 20 and 96%. Diabetes, particularly type-2 diabetics have higher lipid levels than non-diabetics and those patients with poor diabetic control further exaggerate this [2, 3].
Diabetes Mellitus is among the most common non-communicable disease in both developed and developing countries. It is a major cause of morbidity and mortality. It was listed as the seventh leading cause of death in 2007. Recent estimate suggested that diabetes was the fifth leading cause of death worldwide and was responsible for almost 5.1 million deaths in 2013. Every six seconds a person dies from diabetes.
The Worldwide prevalence of diabetes mellitus is expected to rise from current estimate of 422million in 2015 to more than 642 million by 2040. India had 65.1 million people with type 2 diabetes. The prevalence in the Southern part of India was found to be higher at 13.5% in Chennai, 12.4% in Bangalore and 16.6% in Hyderabad compared to Eastern India (Kolkata) 11.7%, Northern India (New Delhi) 11.6%, and Western India (Mumbai) 9.3%.
By 2035, the IDF predicts, India will have 109 million people with diabetes making it the "Diabetic capital of the world". Estimated Mean health expenditure for diabetes is 95US$(Rs.6270/-) in 2015 [4-7].
Therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. But the currently available drugs are associated with side effects. Hence the search is made for new compounds with multiple targets and without any side effects.

There are numerous traditional medicinal plants reported to have antidiabetic and hypolipidaemic properties [8-10]. Gymnema sylvestre is a woody plant found in tropical forests of India and Africa has been used as antidiabetic drug [11]. The medicinally active parts of the plant are the leaves and the roots although the exact mechanism is unknown. Besides impairing the ability to discriminate sweet taste, increased enzymatic activity is responsible for the glucose uptake and utilization. It may stimulate pancreatic β-cell function, increase β-cell number and increase insulin release by increasing cell permeability to insulin. It has additive effect when used concomitantly with hypolipidemic agent [12, 13].
In the present study research was done to evaluate the antidiabetic and hypolipidemic activity of aqueous leaf extract of Gymnema sylvestre in alloxan induced diabetic rabbits.

Material and Methods

The study was conducted at Department of Pharmacology, Gandhi Medical College. Ethical clearance was obtained from Institutional Animal Ethics Committee, Gandhi Medical College, Secunderabad on 20/04/2013, before conducting the experiment.

Extract of Gymnema sylvetre

Aqueous leaf extract of Gymnema sylvetre (sample-ORG-117DE/AHOGS-1303/ref.no.SP-116) was obtained from Himalaya Drug Company, Bangalore, India.

Experimental Design

Healthy adult male New Zealand albino rabbits weighing 1500 to 2500 g were obtained from the central animal house of Gandhi Medical College, Secunderabad with CPSCEA registration no.428/1/C, date 20/6/2001. They were housed in appropriately labelled steel cages according to groups (1 per cage) in a room maintained at 12 hour light-dark cycles and at a constant temperature of 24±2°C and acclimatized for one week before experiment. The animals were fed green lusan, fresh water ad libitum.
Diabetes was induced by injecting 100-120 mg/kg body weight of Alloxan Monohydrate dissolved in 8ml of distilled water intravenously into the marginal ear vein. 2 grams of glucose/kg body wt dissolved in 10 cc of distilled water, was administered orally to each rabbit prior to alloxan injection and 4ml of 25% dextrose is administered i.v after alloxan injection to minimize the anticipated Alloxan Induced hypoglycemia [14]. After 8 days, the rabbits having a FBS of more than 200 mg/dl were considered as diabetic. (normal blood glucose levels in rabbits:100-145mg/dl) [15, 16].
Rabbits were randomly divided into three groups, each comprising six Rabbits and treated as follows:

Group 1: Normal healthy control-distilled water;
Group 2: Diabetic Rabbits-Gymnema sylvestre extract (800mg/kg/po);
Group 3: Diabetic rabbits-Glibenclamide (0.5mg/kg/po) for 30 days.

Dose selection

Glibenclamide: In the present study the dose of glibenclamide 0.5mg/kg body weight per oral was selected. The daily dose of Glibenclamide for albino rabbits was calculated by extrapolation from the human dose (10mg/day).
Human to rabbit dosage conversion of Glibenclamide

10mg/70kg × 0.07 = 0.7mg/1500gm of rabbit [17]

Method of Preparation of Glibenclamide Suspension
The stock solution was prepared by dissolving 7.5 mg of Glibenclamide in 15 ml of distilled water and administered as a standard drug in a dose of 0.5 mg/kg body weight for the standard group.

Gymnema sylvestre: In the present study the dose of 800 mg/kg was selected. The dose was selected based on the reports in previous study which had antidiabetic and hypolipidemic activity [13]. All doses were administered between 9 - 9:30am.
Method of Preparation of gymnema sylvestre Suspension
The stock solution was prepared by dissolving 9 gm of gymnema sylvestre extract in 18 ml of distilled water and administered as a test drug in a dose of 800 mg/kg body weight for the test group.

Blood Sampling: All blood samples were collected within half an hour period between 9:00 am and 9:30 am. Twelve hours fasted blood samples were collected from rabbit's ear marginal vein/central artery.
Blood samples were collected for Blood glucose estimation and for Serum lipid profile estimation on day 0 and 30.

Statistical Analysis

Statistical analysis was carried out using Graphpad Prism 5.0. All results were expressed as mean ± standard deviation (SD). Groups of data were compared with analysis of variance (ANOVA) followed by Post – hoc Tukey's Multiple Comparison Test. $P < 0.05$ was considered statistically significant.

Results

In the present study rabbits were divided in to three groups. Group 1 were healthy controls and received distilled water, Group 2 & Group 3 were diabetic rabbits (alloxan induced),the former received Gymnema sylvestre (800mg/kg) and the later, the standard drug Glibenclamide (0.5mg/kg/day) for 30 days.

Calculation of Percentage Change in FBS & lipid levels

Percentage change from initial values (day 0) calculated on day 30 of treatment period.

$$\text{Percent Change (\%) on day30} = \frac{day\ o\ serum\ levels - day\ 30\ serum\ levels}{day\ 0\ serum\ levels} \times 100$$

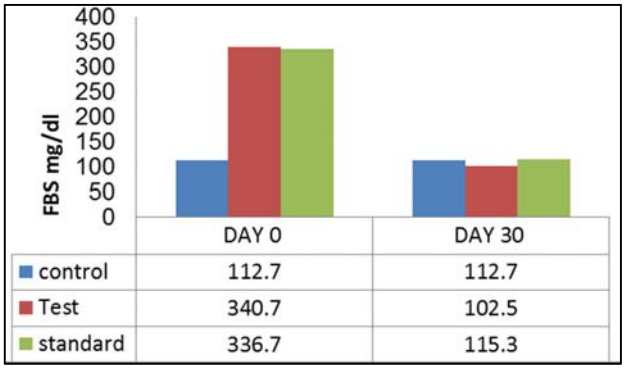
When the blood glucose levels were analysed on day 30, both the diabetic groups showed a significant reduction in blood glucose levels when compared to control group.
When the lipid levels were analysed on day 30, reduction in TC, TG, except LDL levels and increase in HDL levels in between Group 2 and Group 3 were statistically significant.
For all lipid parameters there was no significant difference between Normal control and Group 2 treated with Gymnema sylvestre (800mg/kg/day).
The present study showed 69.9% change in blood glucose levels; percentage change in lipid profile were TC-38%; TG-30%; HDL-12.30%; LDL-78.78% after 30days of treatment with aqueous leaf extract of Gymnema sylvestre(800mg/kg) in alloxan induced diabetic albino rabbits.{Table.1}
For TG and HDL levels there was a significant difference whereas for TC and LDL levels there was no significant difference between Normal control and Group 3 treated with Glibenclamide (0.5mg/kg/day).{Table-2}. Day wise percentage changes in blood glucose and lipid profile has been shown in bar diagrams and line diagrams.

Table 1: FBS and Serum Lipid Profile in Different Groups expressed as Mean+/- SD, F value and P value (ANOVA) followed by Post-hoc Tukey's test.

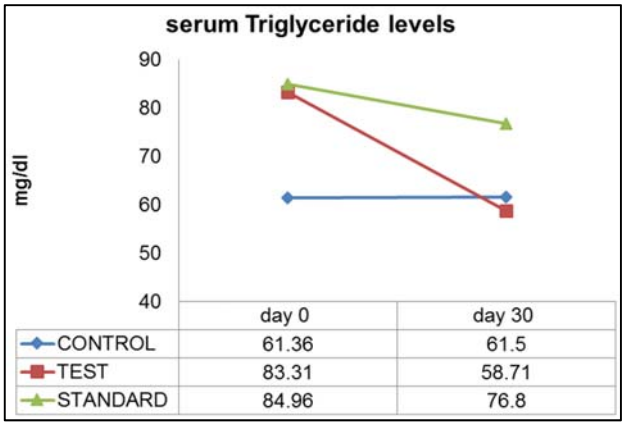
		Control	Test	Standard	F value	P value
Day 0	FBS	112.7 ± 6.77	340.7 ±7 1.52	336.7 ± 71.99	29.63	<0.0001***
	TG	61.36 ± 8.15	83.32 ±11.79	84.96 ± 16.61	6.491	0.0093**
	TC	45.22 ± 5.83	71.26 ±11.25	63.53 ± 11.52	10.98	0.0012**
	HDL	27.78 ± 0.78	23.15 ± 1.86	22.91 ± 1.574	20.68	0.0001***
	LDL	5.915 ± 2.67	31.44 ± 10.5	23.94 ± 9.42	14.97	0.0003***
Day 30	FBS	112.7±7.39	102.5±4.51	115.3±8.26	5.76	0.0139**
	TG	61.5 ± 6.77	58.71 ± 7.17	76.8 ± 8.66	9.913	0.0018**
	TC	47.83 ± 5.27	44.08 ± 4.81	52.41 ± 2.84	5.279	0.0184*
	HDL	27.2 ± 1.07	26.01 ± 1.35	23.93 ± 0.82	13.46	0.0004***
	LDL	8.333 ± 4.80	6.672 ± 4.71	13.14 ± 4.13	3.26	0.0664

Table 2: FBS and Serum Lipid Profile in Different Groups: Post-hoc Tukey's Multiple Comparison Test

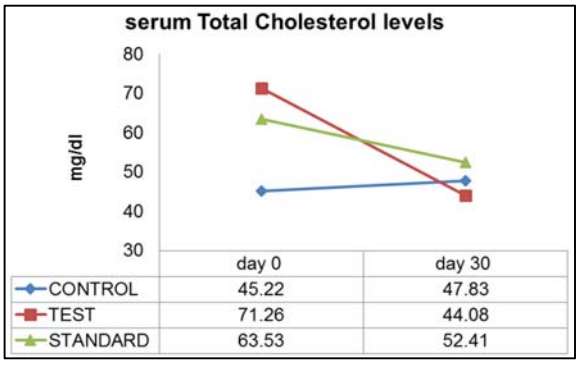
Groups		Day 0	Day 30
FBS	Control Vs Test	9.511***	3.604
	Control Vs Standard	9.344***	0.9453
	Test Vs Standard	0.1669	4.549*
TG	Control Vs Test	4.246*	0.9024
	Control Vs Standard	4.563*	4.946**
	Test Vs Standard	0.3168	5.848**
TC	Control Vs Test	6.452**	2.069
	Control Vs Standard	4.536*	2.519
	Test Vs Standard	1.916	4.588*
HDL	Control Vs Test	7.675***	2.642
	Control Vs Standard	8.064***	7.248***
	Test Vs Standard	0.3891	4.606*
LDL	Control Vs Test	7.528***	0.8937
	Control Vs Standard	5.315**	2.586
	Test Vs Standard	2.213	3.480



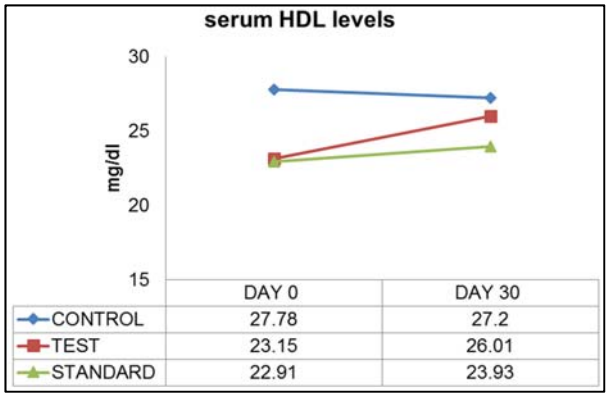
Bar diagram 1: showing day wise variation in blood glucose levels in various study groups. Y-axis represents blood glucose levels.



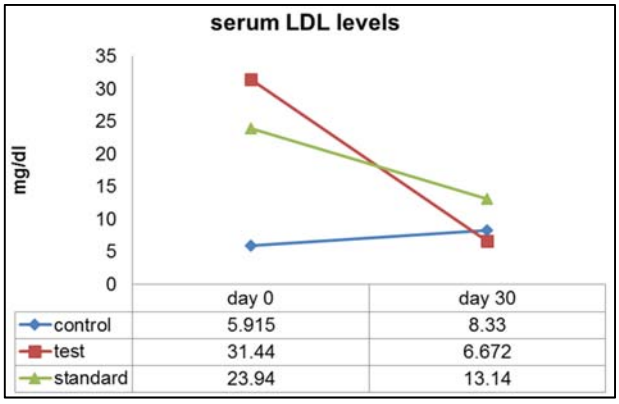
Line diagram 1: showing before and after treatment variation in seum TG levels in various study groups. Y-axis represents seum TG.



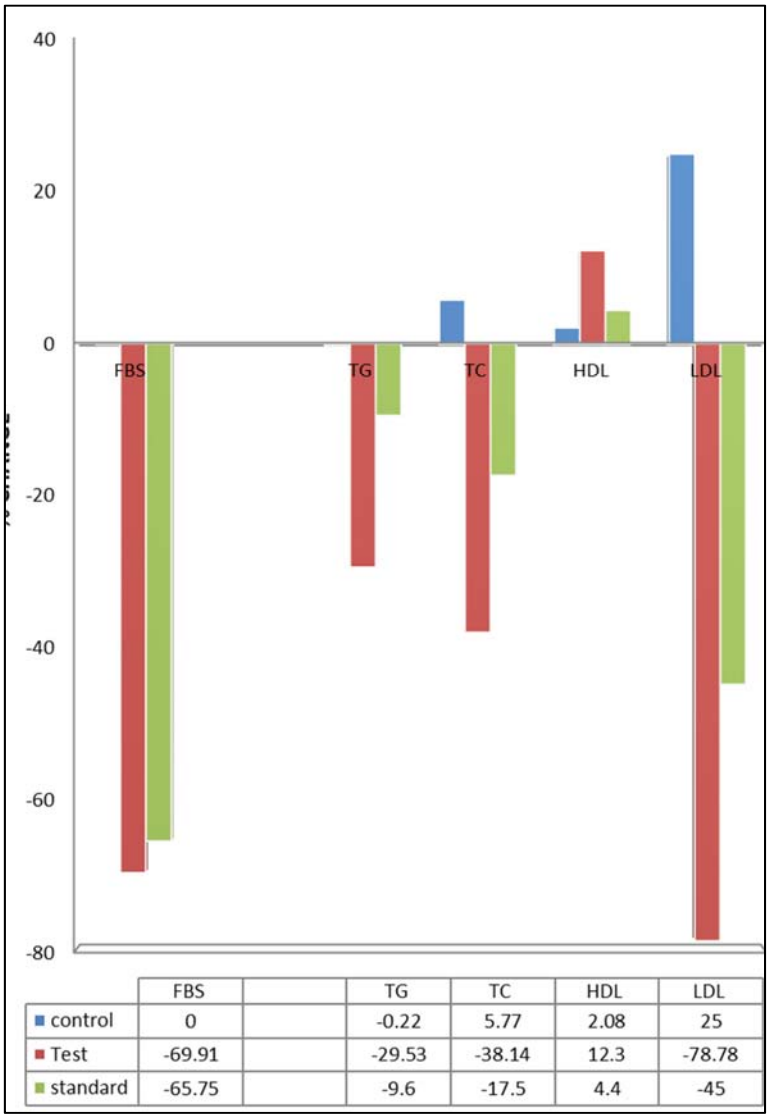
Line diagram 2: showing before and after treatment variation in Serum Total Choleterol levels in various study groups.



Line diagram 3: showing before and after treatment variation in Serum HDL levels in various study groups. Y-axis represents Serum HDL Levels.



Line diagram 4: showing before and after treatment variation in seum LDL levels in various study groups. Y-axis represents Serum LDL Levels



	FBS		TG	TC	HDL	LDL
control	0		-0.22	5.77	2.08	25
Test	-69.91		-29.53	-38.14	12.3	-78.78
standard	-65.75		-9.6	-17.5	4.4	-45

Bar diagram 2: Percentage change in FBS and LIPID levels in various groups over the treatment period

Discussion

Diabetes Mellitus is a spectrum of common metabolic disorders, arising from a variety of pathogenic mechanisms, all resulting in hyperglycemia. The number of individuals with diabetes is rising rapidly throughout the world. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities. However, the major sources of the morbidity of diabetes are the chronic complications that arise from prolonged hyperglycemia, including retinopathy, neuropathy, nephropathy, and cardiovascular disease [18]. Decreased glucose uptake causes increased fatty acid oxidation and release of free fatty acids [19].

In a study of the significance of blood lipid alterations in diabetes mellitus, plasma triglyceride and cholesterol levels in a large series of diabetic and non-diabetic subjects of all ages were measured [20]. Their results showed that plasma triglycerides increase with age in diabetics but not in non-diabetics, while cholesterol levels increase with age in both groups. Thus it is seen that extensive work has been carried out over the decades regarding the hyperlipidemia in diabetics. Dyslipidemia is a relatively common problem in patient with poorly controlled diabetes mellitus. It has been estimated that the frequency of elevated plasma lipid levels in diabetic patients is between 20 and 96%. Diabetes, particularly type-2 diabetics have higher lipid levels than non-diabetics and those patients with poor diabetic control exaggerate this [2, 3].

Therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. But the currently available drugs are associated with side effects. Hence the present study aimed to evaluate the antidiabetic effect of leaf extract of Gymnema sylvestre in comparison with Glibenclamide in “Alloxan induced diabetic rabbit model”.

In the present study rabbits were divided in to three groups. Group 1 were healthy controls and received distilled water, Group 2 & Group 3 were diabetic rabbits (alloxan induced),the former received Gymnema sylvestre(800mg/kg) and the later, the standard drug Glibenclamide (0.5mg/kg/day) for 30 days.

When the blood glucose levels were analysed on day 30, both the diabetic groups showed a significant reduction in blood glucose levels when compared to control group.

When the lipid levels were analysed on day 30, reduction in TC, TG, except LDL levels and increase in HDL levels in between Group 2 and Group 3 were statistically significant.

For all lipid parameters there was no significant difference

between Normal control and Group 2 treated with Gymnema sylvestre (800mg/kg/day).

For TG and HDL levels there was a significant difference whereas for TC and LDL levels there was no significant difference between Normal control and Group 3 treated with Glibenclamide (0.5mg/kg/day). [table-2]

The present study showed 69.9% change in blood glucose levels; percentage change in lipid profile were TC-38%; TG-30%; HDL-12.30%; LDL-78.78% after 30days of treatment with aqueous leaf extract of Gymnema sylvestre (800mg/kg) in alloxan induced diabetic albino rabbits.

In a similar study on Antidiabetic and hypolipidemic effect of leaf extract of Gymnema sylvestre (800mg/kg) in alloxan induced diabetic rats showed 69% change in blood glucose levels; percentage change in lipid profile were TC-46%; TG-50%; HDL-30% after 30days of treatment [13].

Another study "Gymnema sylvestre (500mg/kg) suspension cell extract showed antidiabetic potential in Alloxan induced diabetic male albino rats" showed 46% change in blood glucose levels after 21days of treatment [12].

The another research reported that leaf and callus extracts of *G. sylvestre*, significantly increase the body weight, liver, pancreas and liver glycogen content in alloxan-induced diabetic rats (Wistar rats). The gymnemic acid of leaf and callus extracts significantly increases the regeneration of β-cells in treated rats, when compared with the standard diabetic rats [21].

Gymnema sylvestre leaf extract, the peptide 'Gurmarin', has been found to interfere with the ability of the taste buds on the tongue to taste sweet and bitter. Gymnemic acid has a similar effect. It is believed that by inhibiting the sweet taste sensation, people taking it will limit their intake of sweet foods and this activity may be partially responsible for its hypoglycemic effect [22].

The possible mechanisms by which the leaves and especially Gymnemic acids from Gymnema sylvestre exert its hypoglycemic effects are:

1) it increases secretion of insulin,
2) it promotes regeneration of islet cells,
3) it increases utilization of glucose: it is shown to increase the activities of enzymes responsible for utilization of glucose by insulin- dependent pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase, and
4) it causes inhibition of glucose absorption from intestine. [23]

Gymnema sylvestre is used as an antidiabetic drug in patients with type 2 diabetes mellitus in Ayurveda, branch of alternate medicine.

To obtain the final assessment of antidiabetic activity of Gymnema sylvestre large scale clinical trials of herbal medicine, Gymnema sylvestre are necessary as per ICMR guidelines.

Conclusion

In this study, antidiabetic effect of leaf extract of Gymnema sylvestre was excellent and equally effective when compared with Glibenclamide. There was significant hypolipidemic effect observed with leaf extract of Gymnema sylvestre.

Hence it is promising antidiabetic agent for the development of a phytomedicine for diabetes mellitus. Hypolipidemic effect of Gymnema sylvestre extract should be further evaluated in comparison with standard hypolipidemic drugs.

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Declarations

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Conflict of interest: None declared
Ethical approval: Reg.no:428/01C/CPCSEA

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