

An Assessment of Diabetes Amelioration Potentiality of Whole Extract of *Heliotropium indicum* in a Diet Mixed Dose-Dependent Approach

Abstract

Context: So many plants are reputed to possess diabetes ameliorating potentiality from ancient times. Some of those are proven as anti-diabetic. In contrast, the therapeutic potentiality of others is evidenced as either baseless or has not been proven yet under the light of in vivo analysis. *Heliotropium indicum*, grows by the roadside and wildy is also considered as anti-diabetic species in complementary and alternative treatment practice.

Objective: The aim of this study was to ascertain the therapeutic effects of *Heliotropium indicum* whole extract and figure out its safety profile to ameliorate diabetic conditions using the plant material.

Materials and methods: Dried powder of *Heliotropium indicum* was dipped in ethanol. Next, extract was gathered after rotary evaporation. Alloxan monohydrate induced diabetic rats were treated with extract in a diet mixed manner. [Include statements regarding your use of other antidiabetic medicaments for comparison]

Results [Summarize the results please]

Discussion: Blood glucose levels were measured in both diabetic and non-diabetic rats over the course of the study. Upon measurement of blood glucose levels, all doses of *Heliotropium indicum* were found to decrease the elevated blood glucose levels in rats. However, only 750 mg/kg ($p < 0.05$) reduces blood sugar levels. Thus, the average dose delivered the highest efficiency due to higher food and dose consumption

Conclusion: The study concluded that metformin and leaf extract improved the diabetes-induced medical condition. It essentially shows that *Heliotropium indicum* extract could be used as a significant therapy for treating diabetes.

Keywords: *Heliotropium indicum*, blood sugar, extract, alloxan, in-vivo

Introduction

Diabetes mellitus is a group of autoimmune, metabolic, and genetic illnesses that all have one fundamental feature: hyperglycemia. It is the umbrella term for a broad range of metabolic illnesses **that are** characterized by chronic hyperglycemia[1]. It caused a 3% rise in adult death rates between 2000 and 2019 **[in which country or do you mean globally?]**. Diabetes-related mortality increased by 13% in lower-middle-income nations [2].

Sulfonylureas, biguanides, -glucosidase inhibitors, thiazolidinediones, and non-sulfonylurea secretagogues are examples of oral hypoglycemic medications [3]. Several drawbacks to using these oral hypoglycemic medications have been identified, including drug resistance (reduced effectiveness), side effects, and even toxicity.

There has been a surge in scientific interest in medicinal plants that have historically been used to treat diabetes in humans. This is due to the enhanced efficacy of novel plant-derived therapies, rising interest in natural goods, and the existence of major adverse effects associated with traditional pharmaceuticals[4].

Gynura procumbens, *Terminalia chebula*, *Coccoloba indica*, *Coccoloba grandis*, etc. medicinal plants have direct effects on insulin secretion, activation of glycogenesis and hepatic glycolysis, potassium channel blocker activity, cAMP activation, and modulation of glucose absorption from the intestine and all show antidiabetic effects [5–7]. Presence of alkaloids, saponins, phenols and anthraquinones in the plant *Heliotropium Indicum L.* also proved to give antidiabetic properties [8]

Heliotropium indicum, locally known as Hatishur belonging to the family Boraginaceae, has been used for centuries as a traditional medicine in many nations [9,10]. It is an annual or perennial herb with a pleasant scent and can reach 80 centimeters in height that is native to Asia [10,11].

Numerous researchers have been looking into the phytochemical and pharmacological properties of *H. indicum* to identify the compounds that have led to its widespread use as a herbal medicine [1]. Hatishur has been valued for its medicinal properties to treat a wide range of illnesses in various folk by traditional healers.[11,12]. Alkaloids, triterpenes, sterols, amines, volatile oils have been found as constituents of the plant. Helindicine, a brand-new pyrrolizidine alkaloid, has also been isolated from its roots [9,13]. The plant is said to have antinociceptive, antibacterial, uterine stimulant, febrifuge antifertility, wound healing, anti-inflammatory, antitumor, diuretic and menstruation activator properties. Additionally, it is said to have antifertility, wound healing, and wound healing activities, even though the plant takes many days to get rid of them [13,10].

In our study, we had intended to explore whether our plant possesses anti diabetic activity or not. Consequently, we have measured biomedical parameters to assess the overall physiological state of rats and side effects of the plant on rats. Diet mixed therapy had been employed in this regard to carefully observe the intake of the plant by rats and whether it could contribute to the control Diabetes mellitus or not. Our aim was to assess the therapeutic efficiency of *H. indicum* against Diabetes mellitus. This may provide scientists with sufficient grounds to explore the constituents of the plant in order to control the disease.

Materials and Methods

Drugs, Chemicals and Instruments

Alloxan and ethanol were purchased from Sigma-Aldrich, Germany. Metformin, a standard antidiabetic drug was obtained as a gift sample from Healthcare Pharmaceutical Limited. Plasmatic Laboratory Product Ltd. in the UK provided HDL, LDL, Triglyceride, Total Cholesterol, SGOT, SGPT, and Creatinine. The glucometer Alere GI of Alere Inc. USA, was purchased from Shahbag in Dhaka, Bangladesh, and the Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer) was utilized to evaluate the biochemical parameters.

Plant Collection and Extract Preparation

The whole *H. indicum* plant was gathered from the University of Dhaka's Faculty of Pharmacy's medicinal plant garden.

Before being coarsely pulverized, the plant components were sun dried for 7 days to 10 days. The powdered leaves were violently shaken for 120 hours while steeping in 75% ethanol. After the extract had finished soaking, it was filtered, and the filtered liquid was gathered. After that, a rotary evaporator was used to concentrate the extracted solution. The dried extract was then meticulously collected and preserved for further use.

Experimental Animal handling

Adult, healthy male Wistar rats weighing between 125 and 200 grams were obtained from the Pharmacy department of Jahangirnagar University in Dhaka, Bangladesh, and housed at the Institute of Nutrition & Food Science, University of Dhaka, where they were maintained in a temperature-controlled environment at 25 degrees Celsius with a 12-hour light/dark cycle. Regular supplies of a standard pellet diet and pure water were given. Before the inquiry began, the rats were left there to acclimate. The experiments involving rats were all conducted in accordance with the institutional animal ethics committee's rules (IEAC). According to the standards of the Swiss Academy of Sciences (SCNAT) and Swiss Academy of Medical Sciences (SAMS), animals were handled and treated humanely.

Experimental Guidelines

The 2013 Declaration of Helsinki's ethical guidelines were followed in the execution of all tests.

Dose Selection

Before the study officially started, a pilot analysis was conducted. This analysis revealed that the plant extract (*H. indicum*) began to have a pharmacological effect at a dose of 600 mg/kg, indicating that a dose larger than 500 mg/kg would result in the MEC (Minimum Effective Concentration) value. This impact was seen to steadily increase as the dose was raised. When the dose was eventually increased from 1200 mg/kg to 1600 mg/kg, no discernible change in the pharmacological effects was found. This demonstrated that at a dose of 1200 mg/kg, the plant's pharmacological action related receptors started to become saturated.

Experimental design

Analysis of anti-hyperglycemic activity (Table 1), whereas per their body weight an even distribution of rodents had taken place, and each group comprised of 10 rats.

Table 1: Anti-hyperglycemic Activity Analysis.

Group Number	Group Status	Treatment Specimen	Dose of Treatment specimen (mg/kg)	Group Abbreviation
1	Control	N/A	N/A	C
2	Alloxan Control	N/A	N/A	A
3	Alloxan + Metformin	Metformin	100mg/60Kg	A+M ₁₀₀
4	Alloxan + Metformin	Metformin	200mg/60Kg	A+M ₂₀₀
5	Alloxan + Metformin	Metformin	400mg/60Kg	A+M ₄₀₀
6	Alloxan + <i>H.indicum</i>	<i>H.indicum</i>	600 mg/Kg	A+H ₆₀₀
7	Alloxan + <i>H.indicum</i>	<i>H.indicum</i>	900mg/Kg	A+H ₉₀₀
8	Alloxan + <i>H.indicum</i>	<i>H.indicum</i>	1200 mg/Kg	A+H ₁₂₀₀
9	Metformin	Metformin	100mg/60Kg	M ₁₀₀
10	Metformin	Metformin	200mg/60Kg	M ₂₀₀
11	Metformin	Metformin	400mg/60Kg	M ₄₀₀
12	<i>H.indicum</i>	<i>H.indicum</i>	600 mg/Kg	H ₆₀₀

13	<i>H.indicum</i>	<i>H.indicum</i>	900mg/Kg	H ₉₀₀
14	<i>H.indicum</i>	<i>H.indicum</i>	1200 mg/Kg	H ₁₂₀₀

In **Table 3**, the alloxan control group **indicated** that **the** rats were treated with alloxan only. The N/A **refers to the fact** that no therapeutic treatment was administered to rats of this group.

Induction of Diabetes

In the rat model, alloxan was used to induce diabetes. First, a cold citrate buffer (0.1M, pH=4.5) was used to dissolve alloxan. Then, this alloxan was administered to the rats intraperitoneally at a dose of 150 mg/kg body weight. Within 72 hours of receiving alloxan, it was discovered that all of the rats had an average blood glucose level greater than 13.5 mmol/L, which amply demonstrated their hypoglycemic or diabetic conditions. Blood glucose levels were measured four times daily at a six-hour interval to check for hyperglycemia or diabetes **mellitus**.

Treatment procedure

Respective doses of Metformin and extract of *H. indicum* treatments were given in the rat's cage in a diet mixed manner. Here, **all of the** rats were placed in a single cage. So that estimation of **the dose intake could** easily be evaluated by measuring the leftover. Blood sugar level was assessed once in a week with a treatment window of 6 weeks. Glucometer was used to assay blood glucose level.

Biological sample collection

Rats' tail tips were punctured to obtain blood samples for the measurement of blood glucose levels. Instead, blood was extracted from the sacrificed heart as soon as possible and transferred to a microcentrifuge tube. In order to acquire the supernatant fluid, the collected samples were centrifuged at 5000 rpm for 5 minutes. In order to perform biochemical assays, this fluid was then transferred to another microcentrifuge tube.

Estimation of biological parameters

By using Humaluzer-3000, liver and kidney functioning tests, lipid profiles were performed e.g: HDL, LDL, SGPT and SGOT.

Statistical Analysis

Each group's study variables are shown as mean \pm SD. The "One Way Anova Test" was used to **analyze** the statistical significance of inter-group heterogeneity in terms of various biological characteristics. A software called "SPSS 16" was employed for the analysis. In order for the result to be considered statistically significant, the "p" value obtained must be less than 0.05 ($p < 0.05$).

Result and Discussion

Table 2 below represents the initial and final body weights of the rats in fourteen groups.

Group number	Group status	Body Weight (gm)	
		Initial	Final
1	Control	137.8± 4.54	153.9± 4.47
2	Alloxan control	141.8± 3.46	122.6± 1.85
3	Alloxan + <i>M100</i>	144.8± 4.67	120.8± 5.03
4	Alloxan + <i>M200</i>	137.9± 3.78	117.5± 4.58
5	Alloxan + <i>M300</i>	132.8± 3.73	110.9± 2.41
6	Alloxan + <i>HI600</i>	144.8± 4.54	137.8± 3.19
7	Alloxan + <i>HI900</i>	141.7± 3.62	133.8± 4.66
8	Alloxan + <i>HI1200</i>	142.8± 7.17	136.8± 3.37
9	Metformin <i>M100</i>	137.4± 4.57	130.7± 3.05
10	Metformin <i>M200</i>	146.8± 4.05	139.4± 5.08
11	Metformin <i>M300</i>	149.8± 4.01	141.5± 3.78
12	<i>H. indicum</i> low dose <i>HI600</i>	149.8± 2.34	163.4± 1.06
13	<i>H. indicum</i> medium dose <i>HI900</i>	135.8± 3.41	142.9± 3.28
14	<i>H. indicum</i> high dose <i>HI1200</i>	145.7± 2.38	158.4± 1.44

From the data in Table 2, it has been observed that the rats in group 1 (control group) had a visible weight gain due to increase in their age and body mass. There has been a significant and rapid reduction of rat body weight in group 2 following alloxan administration since it exerted damaging histological and metabolic effects. Groups 3–5 where API metformin had been applied alongside alloxan depicted more severe weight loss when compared to that of group 2. Groups 6–8 also depicted weight loss but at a lesser rate as plant extract may hinder the effect of alloxan. In groups 9–11 where metformin only had been administered present weight loss to a lesser degree, while groups 12–14 where the *H. indicum* plant extracts had been administered in low, medium and high doses respectively showed a visible increase in rat body weight.

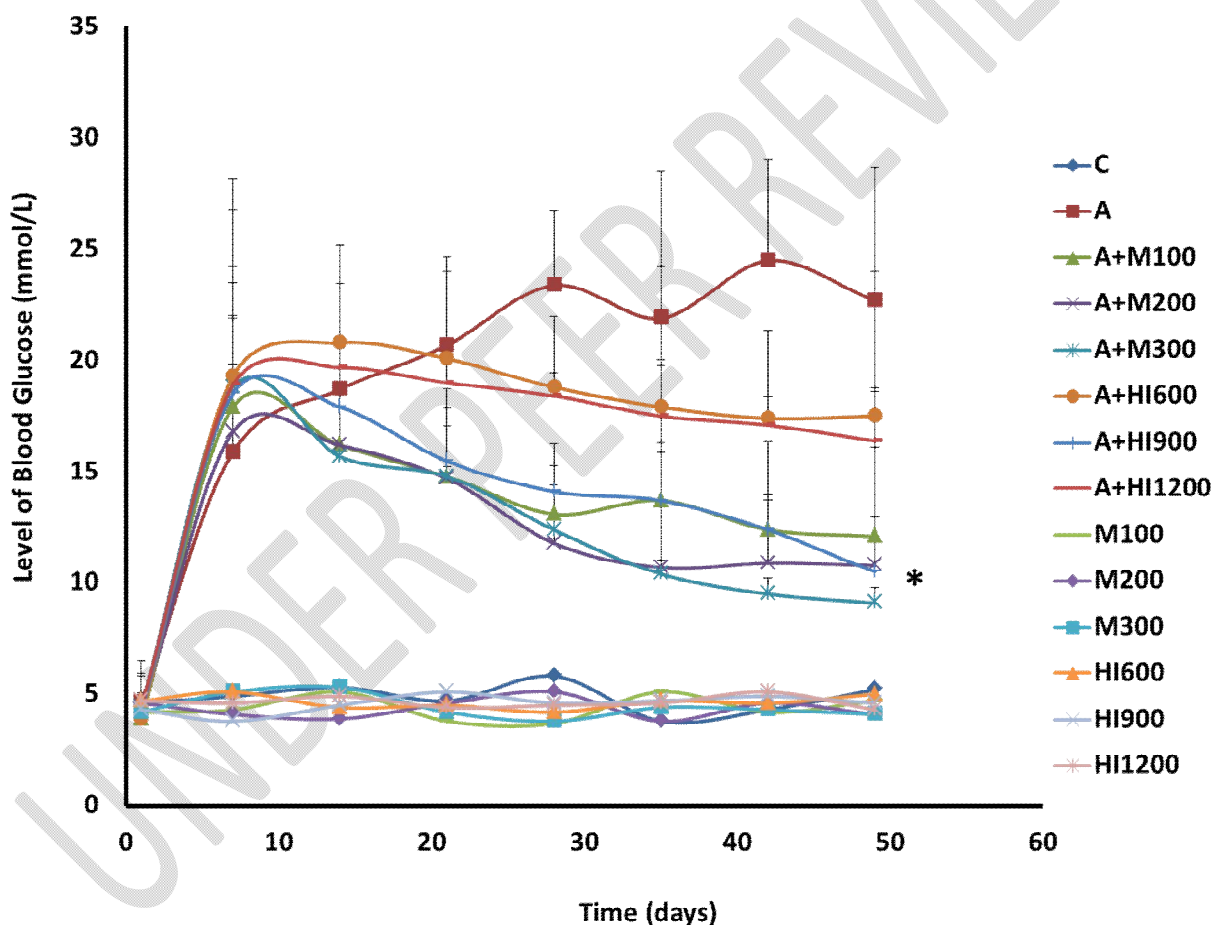


Figure 1 Evaluation of anti-hyperglycemic activity

Plasma glucose concentration of 140 rats in 14 separate groups studied over the course of treatment. The values were presented as the mean \pm SD (n = 10/group). * $p < 0.05$ and indicated

significant difference from the disease group (C=control group, A=alloxan-treated group, M=metformin, A+M = alloxan + metformin, A+HI = alloxan+ *H. indicum*, and HI= *H. indicum* plant extract only

To determine how *H. indicum* extracts exerted their effect on plasma glucose concentration, 140 rats had been used to perform the procedure. They were divided into 14 groups on the basis of their medication and dose administered. Before administering alloxan to the rats in groups 2–8, the initial blood glucose levels of all the rats had been measured. Diabetes had been induced within three days. Within 72 hours after starting alloxan therapy, it was discovered that all of the rats had an average blood glucose level greater than 15 mmol/L, which amply demonstrated their hyperglycemic or diabetic conditions. The rats remained untreated for the next 1 week.

Then, on day 8, the therapy was started. The medications and dosages were administered to the rats in groups 3-14 for 6 weeks. Both Metformin and plant extract had been mixed with food at their respective doses and given to the rats in groups 3-14. Here, alloxan were injected in rats that belonged to group 2-8. However, alloxan had not been administered to groups 9-14.

The alloxan-control group showed a continuous rise in blood glucose concentration (around 25 mmol/L) till day 49, this occurred mostly due to extensive β -cell destruction. Groups 6 and 8 initially showed a rise in blood sugar level, following a steady decrease. Groups 3, 4 and 5 also showed similar results where the sugar level fell sharply to 10 mmol/L following alloxan and metformin administration. The change in Group 7 was statistically significant ($p < 0.05$) while the group 6 and 8 were not. It was found that the medium dose (900 mg/kg) was more effective than that of the high dose (1200 mg/kg). In HI1200 group, food consumptions by the rats were lower and an amount of leftover food was observed to be higher, perhaps due to bitterness or unpalatability, hence lower activity was observed. In groups 9-14, no significant changes were observed when compared to group 1, the blood glucose concentration was maintained within a fairly narrow range, almost static at around 5 mmol/L as seen in the graph. This indicated that *H. indicum* extracts prevent rise in blood sugar, and they possess anti-hyperglycemic properties.

The results were independent of the environment and identical, comparable results had been obtained from studies regarding [complete the sentence]

Table 3

Group no.	Group status	Kidney Function test		Liver Function test		Lipid Profile Tests			
		Creatinine (mg/Dl)	Urea (mg/dL)	SGOT (μ g/L)	SGPT (μ g/L)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
1	Control	0.51 \pm 0.12	25.51 \pm 2.21	45.93 \pm 4.14	37.8 \pm 4.85	103.12 \pm 4.48	66.03 \pm 3.01	35.94 \pm 1.43	70.75 \pm 2.98

2	Alloxan control	3.21± 0.92	79.72± 6.05	90.02± 6.75	74.82± 4.98	184.34± 5.03	118.48± 4.73	71.59±4.78	36.15±3.86
3	Alloxan + M100	2.21± 0.75	70.18± 3.82	80.08± 2.05	65.9± 2.95	148.97± 7.97	110.66±4.04	62.56±2.93	48.22±4.43
4	Alloxan + M200	1.66± 0.65	65.73± 3.43	77.28± 2.22	57.9± 5.84	132.91± 5.82	104.31± 4.25	56.76± 2.62	58.74±4.93
5	Alloxan + M300	0.93± 0.45	60.61± 3.91	72.3± 3.25	44.62± 6.55	114.77± 7.05	96.35± 14.98	46.31± 5.66	65.20± 2.68
6	Alloxan + <i>H. indicum</i> HI600	2.62± 0.65*	75.33± 2.73	85.35± 3.73	71.72± 4.53	176.79± 4.25	114.21± 4.13	69.56±3.58	39.02±2.31
7	Alloxan + <i>H. indicum</i> HI900	1.10± 0.13*	70.64± 1.80*	79.75± 4.07*	59.52± 2.85*	161.07± 5.67*	108.96± 3.45*	59.64± 2.59*	56.20±5.20*
8	Alloxan + <i>H. indicum</i> HI1200	1.51± 0.32*	76.53± 2.51	85.76± 3.87	69.88± 3.46	177.65± 2.62	113.6± 5.13	66.70± 2.96	42.02±4.56
9	Metformin M100	0.62± 0.12	26.18± 2.91	44.94± 3.10	33.58± 3.70	102.05± 4.06	69.18± 2.10	36.55± 2.68	73.27± 1.48
10	Metformin M200	0.42± 0.48	26.92± 2.82	44.69± 3.94	40.52± 2.35	100.33± 3.34	68.66± 2.01	33.36± 2.37	74.11± 2.28
11	Metformin M300	0.75± 0.25	26.78±3.32	48.85± 2.96	41.94± 3.14	103.46± 5.37	64.00±5.35	35.45±4.46	71.29±4.29
12	<i>H. indicum</i> HI600	0.82± 0.42	27.06±2.41	45.44± 2.83	38.56± 3.16	100.37±3.70	64.90±3.69	39.35±2.38	73.43±4.98
13	<i>H. indicum</i> HI900	0.61± 0.32	26.47±2.63	42.22±3.07	38.45± 2.45	100.33± 4.06	71.45±2.13	39.67±1.77	68.92±4.14
14	<i>H. indicum</i> HI1200	0.74± 0.42	27.13±1.32	43.69± 2.04	41.46± 2.95	102.24±2.80	66.99± 2.20	39.20±2.70	69.26±2.42

Table 3: Values are given as mean ± SEM for 14 groups of rats each *p<0.05. The extract treated groups were compared with the diabetic control.

From table 3, the highest level of serum creatinine had been observed in group 2 due to the kidney damaging action of alloxan. However, groups 3-8 showed statistically significant (p<0.05) deviation in the values of creatinine and group 9-14 showed a comparatively lower values of creatinine, the data being statistically non-significant when compared with group 1.

Again, it has been observed that level of urea was significantly decreased in group 7 when compared with group 2 (p<0.05).

Biochemical markers determining hepatic function such as SGOT and SGPT levels were enhanced significantly in alloxan-induced diabetic rats. In groups 3-5, serum SGOT levels decreased compared to that of group 2 while serum SGPT reduced at a steeper rate. Group 6 and 8 showed SGPT levels near to group 2, the changes in group 7 were found to be statistically significant (p<0.05), while groups 9-14 showed results fairly similar to group 1 in case of both SGPT and SGOT levels.

Alloxan administration in group 2 resulted into visibly altered hepatic and renal function. There was no statistically significant difference in the test results obtained between the Metformin groups (9-11) and the extract-treated groups (10-12), the enzyme levels in serum were markedly reduced due to the cytoprotective and anti-oxidant effects of the *H. indicum* plant extract.

The levels of total cholesterol, LDL, and triglycerides were lower in the treatment groups than in the alloxan-controlled groups, since alloxan raised the concentration of these indicators too. According to Table 3, the negative control group's HDL level was determined to be the highest, while the alloxan control was the group that had the lowest and vice versa in case of plasma cholesterol, triglyceride and LDL. Groups 6-8 depicted fairly identical values of plasma cholesterol levels while in groups 9-14 there were no significant changes observed in comparison to the negative control group. The same happened for serum cholesterol, triglyceride and LDL where the plasma levels decreased in groups 3-5 when compared to that of group 2 and the reverse occurred in the case of plasma HDL. In all the lipid profile test parameters including serum LDL, HDL, triglycerides and total cholesterol, groups 9-14 was observed to yield similar results to that of group 1.

The changes in group 7 (Alloxan+ *HI* 900mg/kg) among groups 6, 7, and 8 were statistically significant ($p < 0.05$) all the respective parameters including hepatic, renal function and lipid profile tests. [amend the sentence to convey the exact meaning you want to convey please]

The plant extract had been administered at a dose of 600-1200 mg/kg body weight and contained a variety of different phytochemical constituents; metformin is a single API given in doses of 100-300 mg/kg body weight respectively. Therefore, its anti-diabetic effects will naturally be lower than that of metformin API.

Conclusion:

Based on our findings, it is easy to conclude that the plant can play an important role in diabetic treatment. The low dose food intake by rats in the high dose treated group, on the other hand, suggests that the bitter taste of the extract is difficult to tolerate without appropriate taste masking. As a result, it can be concluded that further research and the precise isolation of anti-diabetic constituents from extract can increase the likelihood of *Heliotropium Indicum* becoming a part of the diabetic management system.

Conflict of Interest

Acknowledgements

The role played by each author

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