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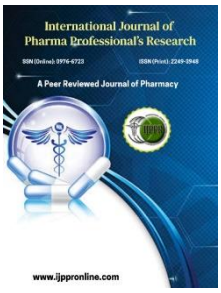

To evaluate the anti-oxidant & anti-diabetic action of Dolichos Trilobus Linn. in Wistar albino rats

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Keywords:
Dolichos trilobus Linn, Beta cells, Antioxidants, Antidiabetics, Streptozocin, Hypoinsulinemia.

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Abstract: Aim: The chief motive for doing the research is to estimate the therapeutic outcomes of aqueous extract of *Dolichos trilobus* to respond diabetes induced by Streptozotocin. **Materials & methods:** The primary focus is to calculate the therapeutic effects of *Dolichos trilobus* given once orally to the two test groups. Thirty Wistar Albino rats were equally circulated between five groups. Study of *Dolichos trilobus* aqueous extract was carried out for 21 days. During this period twelve rats were divided to form two different groups and were given a dose of***"200 mg/kg/day, 400 mg/kg/day"***. No mortality was seen at 400mg/kg even after constantly dosing for next 21 days, considered it safe. Control group was given distilled water on the dose of 10ml/kg/day orally. Streptozotocin was given to both the Negative group as well as Standard group on the dose of 60mg/kg/day via Intra-peritoneal route. Glibenclamide has been set to the Standard group on the dose of 10mg/kg/day orally. Aqueous extract of *Dolichos trilobus* (Sickle Bean) was given to the Test-1 group on the dose of 200mg/kg/day orally. Aqueous extract of *Dolichos trilobus* was given to the Test-2 group at a dose of 400mg/kg/day orally. **Results:** Streptozotocin causes hypoinsulinemia and hyperglycemia due to production of pancreatic islet β-cell destruction. The diabetes was confirmed by increased level of glucose in blood, lipid per oxidation (MDA level), reduction in GSH level, food intake, body weight and histopathological variations were seen in negative group are compared to control group. This evaluation has shown that there was no major diversity in between Standard group and Test-3 (Aqueous extract of *Dolichos trilobus* 200 mg/kg) group. It means Test-3 have nearly same therapeutic efficacy as standard drug. **Conclusion:** The conclusion of the research indicates that the aqueous extract for

Dolichos trilobus L. has antidiabetic action against diabetes induced by Streptozotocin.

1. Introduction

Diabetes is a problem of metabolism of carb, fat or protein, troubling colossal numeral of populaces worldwide. Diabetes is gathering problems of metabolism described via hyperglycemia, considerable issue in the insulin discharge, or insulin movement, sometimes might be all. Raised dehydration, urinary results, ketonemia or ketonuria are the essential signs for diabetes, which emerge on account of anomalies in fat, sugar, and protein digestion.[1]

The quantity of diabetic patients had quickly brought up in the local or transient Asian populations. It creates at a more youthful age in Asian populaces, thusly the bleakness and mortality connected with the illness and its confusions are likewise normal in youthful Asian individuals. It causes a key medical services issue overall and give significant test to patients, and medical care frameworks. According to in the middle of somewhere in the range of 2000 and 2030, the earth populace will raise by 37% and the quantity of individuals with diabetes will support by 114%. Asia is the main spot of a rapidly rising diabetes pestilence. India and China will stay the two nations with the upper most records of individuals with diabetes by 2030.

1.1 Types of Diabetes

It is a bunch of ongoing metabolic conditions, all recognized by expanded glucose level pending around because of body's inadequacy of making insulin, protection from insulin activates, or may be both. This get-together of circumstances could be partitioned in four distinct parts:

i. **Type 1 Diabetes:** It results from beta-cell obliteration in pancreas or distinguished through a complete shy of insulin creation.
ii. **Type 2 Diabetes:** It creates whenever there will be unusually extended protection from the activities of insulin and then the body can't make the adequate measures of insulin to beat that obstruction.
iii. **Gestational type diabetes:** The form of glucose intolerance that affects a few women when they are expecting.
iv. **Set of other types** of diabetes brings about through specific genetic deformity of beta-cells capability or insulin activities, sicknesses in the pancreas, or meds or synthetic compounds.[2]

1.2 Risk Factors of Diabetes

Between 5% and 10% of all cases of diabetes is type-1. Its gamble of factor comprises of the immune system, genetic, and ecological elements. Up till nowadays, there are no known ways of keeping away from Type 1. 90 to 95 percent of all those instances of diabetes that have been studied are type 2 Diabetes. This type of diabetes typically starts as insulin obstruction and, in light of the fact that the body is unfit to make satisfactory insulin to manage the opposition; the pancreas might diminish the creation of insulin or eventually finish making it. Females who are corpulent, or having a family record with diabetes, or gestational type of diabetes in a previous pregnancy could be at more serious gamble comparative to different people for gestational diabetes. Severe glycemic controls and the executives of females with gestational type of diabetes are fundamental as stay away from delivery complexities for the developing newborn child.[3]

1.3 Causes & Symptoms of Diabetes

Reason for diabetes relies upon the sort of diabetes. Reason of Type-1 is a result of beta-cell obliteration, interceded by any of invulnerable intervened or may be idiopathic, while Type-2 happens basically because of insulin obstruction or sometimes along with relative insulin inadequacy. This disease is likewise related to the way of life or hereditary qualities. There are different types of different elements which

attracted advancement of this disease which are hereditary like mitochondrial and chromosomal DNA transformation. Lipoatrophic and Leprechaunism diabetes are related to the hereditary imperfections in the activity of insulin. Now and again intrinsic cytomegalovirus and rubella contamination likewise leads the reason for diabetes. On occasion, meds and different synthetics, for example, nicotinic corrosive, pentamidine, thyroid chemical, glucocorticoids, thiazides, beta-adrenergic agonists, alpha-interferons can prompt diabetes. Anomalies in the pancreas like pancreatectomy, neoplasia, pancreatitis, fibro calculous pancreatopathy and cystic fibrosis, may likewise build up diabetes. Different elements are there which are related to safe framework like 'Firm man' condition and hostile to insulin receptors antibodies. There are so many hereditary disorders like Down condition, Klinefelter condition, Turner disorder, Wolfram disorder, and Friedreich's ataxia.[4]

1.4 Existing therapies for diabetes

Diabetes treatment is an overall issue and fruitful treatment still can't seem to be found. However, oral hypoglycemic and insulin treatment specialists are main treatment for diabetes; they make not show side impacts and neglect the progression of diabetic confusions. Humanoid insulin is polypeptide, eating an atomic load around 6000 Da, comprise of 2-amino corrosive chains A and B, which are coupled by 2 desulphated (- S-) linkages. The typical human pancreas covers around 8-10 mg of insulin. Insulin isn't proper for oral organization as a result of inactivation by stomach-related compounds. 80% of applied insulin is typically debased in the liver, kidneys. The aggregate sum of insulin delivered on a regular schedule in a typical human is surmised 40 units.[5]

1.5. Treatment of Diabetes

1.5.1 Oral Anti-diabetic Drugs

(I) Enhance Insulin Emission

(II) K_{ATP} Channel Blockers

SULFONYLUREAS- They tie to a particular 'Sulfonylurea receptor (SUR1) situated on the pancreatic β cell layer and incite a lively arrival of insulin**.**

- Tolbutamide,
- Glipalamide,
- Glipizide,
- Gliclazide and
- Glimepiride

PHENYLALANINE ANALOGUES- They additionally tie to SUR and closes ATP delicate K+ channels which causes depolarization and further causes insulin discharge.

- Repaglinide, and
- Nateglinide

(III) Inhibitors of DPP-4- They potentiate the activity of Glucagon-like peptide-1 and GLP helps postprandial insulin discharge, diminishes glucagon emission.

- Linagliptin,
- Sitagliptin,
- Alogliptin, and
- Vildagliptin

A) Overcome Insulin Resistance

(I) Biguanide (AMP_k Activator)

Metformin- It stifles hepatic glucogenesis and glucose yield from liver. What's more, it additionally improves insulin-intervened glucose take-up and removal in skeletal muscle and fat. In addition, likewise impedes mitochondrial respiratory chain and advances fringe glucose use through anaerobic glycolysis.

(II) Thiazolidinedione (PPARγActivator)

Pioglitazone- It upgrades the record of a few insulin responsive qualities and will in general opposite insulin obstruction by improving Glucose carrier type 4 (GLUT4) articulations and movement. Because of which passage of glucose into muscle and fat is gotten to the next level. Hepatic glucogenesis is additionally stifled.

B) Retard Carbohydrate Absorption

α- Glucosidase Inhibitors- They reversibly restrains α-glucosidases , the last protein for the assimilation and retention of polysaccharides and sucrose in the brush line of small digestive tract mucosa. Also, GLP1 discharge is elevated which might add with the impact.

- Acarbose,
- Miglitol, and
- Voglibose

C) Miscellaneous

(I) SGLT-2 Inhibitors- Sodium-glucose co-carrier (SGLT) has 2 sorts, SGLT1 and SGLT2. In the kidneys, glucose passes openly from the circulation system into the lumen of the nephron. At the initial segments of proximal tubule, 90% of separated glucose is reabsorbed by SGLT2 through an auxiliary dynamic vehicle with sodium. The leftover 10% is reabsorbed by SGLT1 in the last option part of the proximal tubule. SGLT2 inhibitors block the activity of SGLT2 in the proximal tubule, and glucose is lost in the pee.

- Canagliflozin, and
- Dapagliflozin

(II) Dopamine D2 Agonist- Impacts are interceded by means of resetting of dopaminergic and thoughtful tone inside the focal sensory system (CNS). At the point when regulated in the early morning toward the beginning of the light stage, another fast delivery (QR) plan of bromocriptine seems to act midway to reset circadian rhythms of hypothalamic dopamine and serotonin and further develop insulin opposition, as well as dyslipidemia and glycemic regulator without modification in body weight in type 2 and large non-diabetic people. Bromocriptine appears to function as an amazing asset, switching the unnaturally raised hypothalamic drive for expanded plasma glucose, fatty substances, and free unsaturated fat levels, in fasting and postprandial states in insulin safe patients.

- Bromocriptine.[6]

1.5.2 Anti-diabetic activity shown by herbal/natural plants

Herbal grown drugs are alright for our wellbeing in the treatment of numerous illnesses. However, scarcely any drugs are lesser hurtful or have low viability sun like manufactured ones. That implies we can express that plant-based prescriptions are great in comparision to engineered drugs.

I. ***Acacia arabica***

Acacia Arabica (chloroform extricate) (Leguminosae) when given in diabetic rodents at 250 and 500mg/kg, per oral for a long time, significantly brings down the blood glucose level and complete cholesterol (TC), fatty oil (Tri-Glycerides), or HDL and LDL level were reestablished.

II. *Achyranthes rubrofusca wight*

The ethanolic and fluid concentrate of Rubrofusca (F. Amaranthaceae) leaves in the diabetic rodents were examined against diabetic activities.It diminishes the blood sugar level essentially, the pancreatic protein, for example, glutathione level,superoxide dismutase (Turf) catalase (Feline)were altogether expanded in those treated gathering contrasted with ethanolic extricate.

III. ***Andrographis paniculata Burm. F.***

The organization of the ethanol concentrate of Paniculata (F. Acanthaceae) in the diabetic rodents on a portion of 100 or 200 mg/kg, per oral for 30 dayscontinous treatment, fundamentally diminished the blood sugar level. It reestablished TC, TG, glycosylated hemoglobin, phospholipids, aspartate transaminase, alanine transaminase, soluble phosphate and corrosive phosphate level which demonstrates its enemy of diabetic movement.

IV. *Barleriaprionitis*

Alcoholic concentrates of the leaves and foundation of the Barleriaprionitis (F. Acanthaceae) in those diabetes induced rodents on 200mg/kg, per oral for the next 14 continues days treatment, fundamentally diminished blood sugar and the HbA_{1C} (Glycosylated haemoglobin) levels. In addition liver glycogen and serum insulin level were altogether expanded.[7]

2. Materials and method

2.1 Drugs and chemicals used

Table 1: List of the Drugs and Chemicals used

CHEMICAL NAME	SUPPLIER/CAS NO
DOLICHOS TRILOBUS	AMBE NS AGRO PRODUCT PVT LTD
ETHANOL	(UN NO: 1170) CENTRAL DRUG HOUSE (p) Ltd corp Delhi
CHLOROFORM	Thermo fisher scientific India PVT Ltd 403-404
ACETONE	CHD (CAS NO:67-64-1) CHD
METHANOL	(CAS NO:67-56-1) Central Drug House (p) Ltd corp Delhi
STREPTOZOTOCIN	(cas no:18883-66-4) Central Drug House (p) Ltd crop Delhi
FORMALDEHYDE SOLUTION	(CAS NO:57-11-4) CENTRAL DRUG HOUSE (p) Ltd corp Delhi
POTASSIUM DIPHOSPHATE	Central Drug House (p) Ltd corp Delhi
BOVINE ALBUMIN SERUM	(CAS NO:9048468) CHD
EDTA	(CAS NO:60-00-4) CHD
SODIUM CHLORIDE	FISCHER (CAS NO:7647-14-5)
THIOBARBITURIC ACID (TBA)	CDH (CAS NO:504-17-6)
POTASSIUM CHLORIDE	(Product No. 029594) CHD
COPPER (II) SULPHATE PENTAHYDRATE	(CAS NO. 7758-99-8) CHD
DI-SODIUM TARTRATE	CHD (CAS NO.6106-24-7)
SODIUM DODECYL SULFATE (SDS)	(CAS NO. 151-21-3) CHD

Apparatus and equipments used

Table 2: List of Apparatus and Equipments used

APPARATUS/EQUIPMENT	SOURCE OF ORIGIN
ELECTRONIC BALANCE	**METTLER-TOLEDO INDIA PRIVATE LTD, MUMBAI**
HOMOGENIZER	**REMI, MUMBAI**
LABORATORY CENTRIFUGE	**REMI, MUMBAI**
MICRO PIPETTE	**J. MITRAS & BROS., NEWDELHI**
UV SPECTROPHOTOMETER	**1800, SHIMADZU**

	, USA
WATER BATH	REMI, MUMBAI

2.3 Collection, identification and authentication of plant:

Dolichos trilobus L plant and it is collected from the Bhagvati Augmentin Ayurveda Herbal and Healthcare Pvt. Ltd.

Certificate of analysis is attached BH/18/DB/026/21-22 have been kept in I.T.S. College of Pharmacy, Ghaziabad for future use.

2.4 Animals

All study was performed on spring-up Wistar albino rats weighted 150-300 gm. Animals were obtained by the Animal House of I.T.S College of Pharmacy, Muradnagar, Ghaziabad (U.P.). All the animals were kept up at 23 to 24 oC moistness in a 12-12 hrs day and night cycle with water availability thought while examined access to food. The project proposal number is this (ITS/09/IAEC/2019) and the CPCSEA number is this I044/PO/Re/S/07/CPCSEA, 27th Feb.2007).

2.5 Experimental Design:

(I) Normal Control: This group will be treated as controlled group.

(II Disease Control: Rats will serve as diabetic-control and receive single intraperitoneal (I.P.) injection of Streptozotocin at a dose of60 mg/kgin this group. (**Melanie L Graham,*et al.*, 2011)**

(III) Positive Control: After inducing diabetes with single intraperitoneal (I.P.) injection of 60 mg/kg of Streptozotocin, Glibenclamide will be given for 21 days at a dose of 10 mg/kg/day, p.oin this group.

(IV) Treatment Group I: After inducing diabetes with single intraperitoneal (I.P.) injection of 60 mg/kg of Streptozotocin, *Dolichos trilobus* L. extract will be given for 21 days at a dose of 200 mg/kg/day, p.oin this group. (**Muthu A K*et al,* 2006)**

(V) Treatment Group II Group: After inducing diabetes with single intraperitoneal (I.P.) injection of 60 mg/kg of Streptozotocin, *Dolichos trilobus* L. extract will be given for 21 days at a dose of 400 mg/kg/day, p.oin this group. (**Muthu A K*et al,* 2006)**

2.6 Statistical analysis

All outcomes were showed by Mean ± Standard error of Mean (SEM) in each grouping. Statistical investigations were carried out through utilizing sigma Stat and Sigma Plot statistical software. The significance effect of the distinction between two groups was evaluated via utilizing ***Student's t-test***. For various examinations, one way investigation of difference (ANOVA) was utilized. In case ANOVA was indicated critical distinction, post hoc investigation finished with the Dunnet's test $P<0.01$considered as statistically significant.

2.7 Procedure

i. The bodyweight (B.W.) of individual animals have been taken and recorded daily for every group from the first day of the study and the record was maintained till the last dosing and also before sacrificing the animal.

ii. If any animal dies during the study, its weight will also need to be taken. Food intake (F.I.) will be measured on each day.

iii. All the standard and test drugs are administered for 21 days. Retro orbital puncture method used for blood sample collection at 0th, 7th, 14th, 21st day at 0, 1st, 2nd, 4th, 6th and 8thhr and the GOD-POD kit has been used for blood sugar levels. Plasma lipid profiles and liver enzyme levels were estimated by using biochemical kits on 21st day.

iv. After diabetes has been induced rats become hypoglycemic immediately so we will check the blood glucose at 4, 12, and 24 hr. If the blood glucose level is low,

we will give 5 % DNS to maintain the blood glucose level. All the animals will be sacrificed at the end of the study by administrating 150 mg/kg of Thiopentone sodium 24 h after the final treatment.

v. Ice-cold saline will be used to wash the isolated liver, then will prepare the homogenate and will store at a temp. Of –80°C till further analysis for biochemical assessments and histopathology.

3. Result and analysis

3.1 Valuation of Animal constraints (Body mass & Food Consumption)

Streptozotocin (60 mg/kg) in negative control, notably decreased the body weight and food intake ($p<0.05$) comparative to regular group during 21 days constant action as data enclosed in Figure 1. The decreased Body mass & Food Consumption is greatly increased with all treatment groups. **Table 3:** Valuation of Animal constraints (Body mass & Food Consumption). Streptozotocin (60mg/kg/day), Extract-1 *(*200mg/kg/day) and Extract-2 *(*400 mg/kg/day).

Table 3: Variation in animal's body mass and food consumption

Groups (n=6)	Body Mass (in gms.)	Food Incorporation (in gms.)
Control (Normal)	214.4± 14.73	**80.43± 4.516**
Control (Negative)	156.16±3.08*	**55.36± 2.06***
Standard	204.98 ± 27.94	**79.39± 2.33**
Test 1	181.64 ± 7.6	**59.17± 2.33**
Test 2	**177.45 ± 16.45**	**63.6 ± 2.32***

3.2 Body weight and food intake by the rats

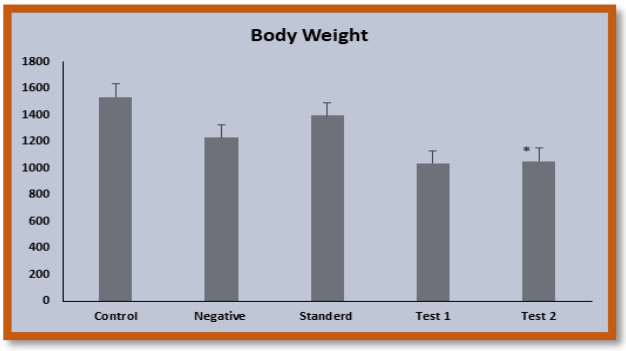

Figure 1: Category-wise body weight distribution in the rats

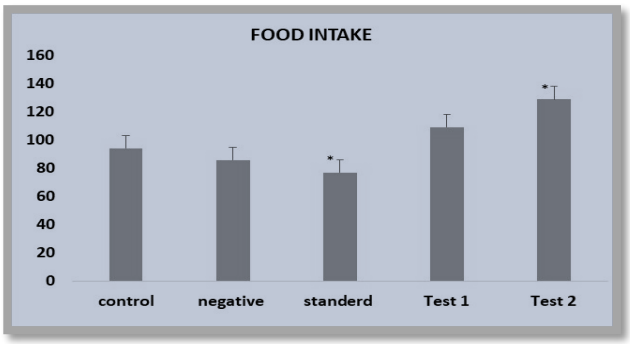

Figure 2: Category-wise food intake chart

3.3 Antioxidant level measurement

The antioxidant echelons are representative in Table 4. Streptozotocin (60 mg/kg) considerably amplified ($p<0.05$) the MDA elevation proportional to the normal groups. The raised level of MDA radically decreased ($p<0.05$) by Test 2 as shown in Figure 3.

Streptozotocin management also decayed the GSH group ($p<0.05$). Test group 2 displayed the substantial refurbishment in the GSH phase within the liver ($p<0.05$) like revealed in Figure 4.

Table 4: MDA and GSH Values in the groups.

Groups (n=6)	MDA (µmolmg^{-1}protein)	GSH (µmol mg^{-1}protein)
Control (normal)	93.5 ± 0.44	**9772.92±492.64**
Control (Negative)	98.31 ± 0.28*	**462.95± 163.45**
Standard	93.47± 0.25	**6274.82± 2448.54**
Test 1	96.67±0.36	**261.79± 550.24**
Test 2	**75.41± 0.231**	**562.85± 219.81**

Figure 3: MDA altitudes in the hepatic tissue of all group animals

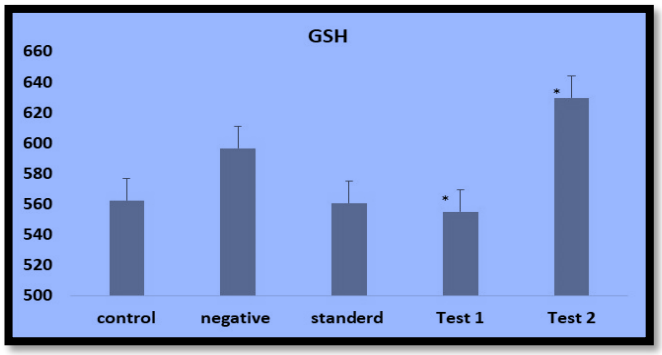

Figure 4: GSH stage in the hepatic tissue of animals

Serum Creatinine, **Blood urea** and **Blood Glucose** levels (**Table 5**) in groups of animals treated by Streptozotocin ***(60 mg/kg/day)***, glimepiride ***(10 mg/kg/day)***, Plant extract 1(200 mg/kg), Plant extract 2(400 mg/kg).

Table 5: Serum Creatinine, Blood urea and Blood Glucose levels

Groups (n=6)	Creatinine	Blood urea	Blood Glucose
Control	0.91 ± 0.26	27.66 ±2.16	146.66 ±6.28
Negative Control	1.08 ± 0.15ns*	31.5 ± 2.42ns*	140.83 ± 0.14ns*
Standard	1.06±0.15ns#ns*	30.5± 2.42ns#ns*	128.33 ± 14.0ns#ns*
Test 1	0.93±0.19ns#ns*	29.33±2.73ns#ns*	133± 015.08ns#ns*
Test 2	0.95±0.15ns#ns*	29.5± 3.27ns#ns*	139 ± 9.69ns#ns*

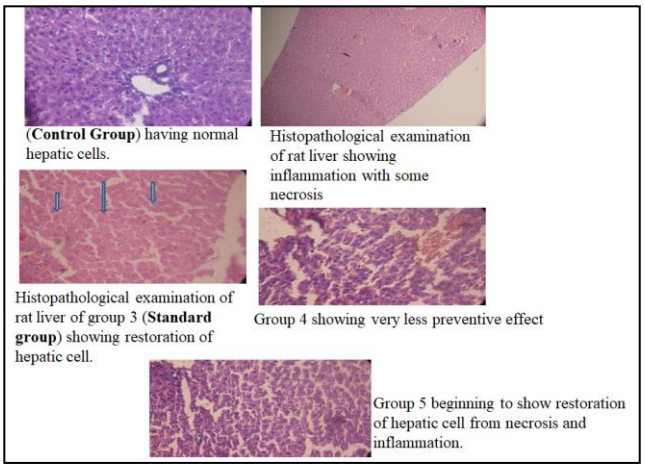

Figure 5: Microscopic examination of the liver cells

4. Conclusion

Detecting the consequence of the current research may accomplish that streptozotocin generates free radicals which are caused by pancreases. Streptozotocin leads to lipid peroxide food intake, body weight, MDA, GSH, creatinine, blood urea, blood glucose. Plant extract (400 ml/kg) was more useful as compared to plant extract (200 mg/kg,) meaningfully diabetes. Due to this study, we can conclude enhance the therapeutic property (antidiabetic) of *Dolichos trilobus* L waste in the treatment of disease like diabetes.

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