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Anti-hyperglycemic activity of leaves extract of *Hyptis suaveolens* L. Poit in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To evaluate the antihyperglycemic activity of leaves of *Hyptis suaveolens* using streptozotocin model. **Methods:** *Hyptis suaveolens* extract (HSE) 250 and 500 mg/kg body weight was administered orally to streptozotocin induced diabetes, once daily for 21 days. **Results:** A significant reduction in blood glucose was observed in diabetic animals treated with HSE at different doses when compared with diabetic rats. Levels of triglyceride, total cholesterol, low density lipoprotein, very low density lipoprotein were decreased while administering HSE at different doses, compared with their control values in diabetic animals. **Conclusions:** Our results show that HSE possesses significant antihyperglycemic activity which might be attributed to stimulating effects on glucose utilization and antioxidant enzyme.

1. Introduction

The concept of developing drugs from plants in indigenous medical system is much older, while in some cases direct links between a local and biomedical use exists, in other cases the relationship is much more complex[1]. *Hyptis* is a genus of Lamiaceae with about 400 species. One of the species from genus, *Hyptis suaveolens* Poit (referred as *Hyptis hereafter*) is naturalized in India[2]. Its distribution is tropical and subtropical and it is not commonly found over 500 m. A plenty of traditional herbal medicinal practices have been used in the diagnosis, prevention and treatment of diabetes. The plant, *Hyptis suaveolens* (L) Poit commonly known as *Wilayati tulsi* is an ethnobotanically important medicinal plant. The plant has been considered as an obnoxious weed, distributed throughout the tropics and subtropics. Almost all parts of this plant are being used in traditional medicine to treat various diseases. Leaves of *Hyptis suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases[3]. Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and antisuporific baths, antiinflammatory, antifertility agents[4] and also applied as an antiseptic in burns, wounds, and various skin complaints. The decoction of roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor[5]. Though there is no scientific evidence to support the antidiabetic effect of *Hyptis suaveolens*, tribal men continue to use the plant in the treatment of diabetes. The objective of this investigation was to ascertain the scientific basis for the use of this plant in the treatment of diabetes using streptozotocin model.

2. Materials and methods

2.1. Plant extract preparation

Dried leaves of *Hyptis suaveolens* (Lamiaceae) were procured from in and around Shevaroy hills, Yercaud in the month of April 2009 and were identified and authenticated by taxonomist of the Botanical Survey of India, Yercaud,

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Tamilnadu, India. The coarsely powdered material was exhaustively extracted thrice with 50% aqueous ethanol, and the yield of *Hyptis suaveolens* extract (HSE) was 9.5% (w/w)

2.2. Animals

Sprague-Dawley rats (150-200 g) and Swiss albino mice (20-25 g) of either sex and of approximately the same age were procured from the animal house of the Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at (26±2)℃ and relative humidity 44%-56% in polypropylene cages. Animals were exposed to alternate 12 hrs of darkness and light each. Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn18-24 h before the experiment though water was allowed *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the regulatory body of the government.

2.3. Chemicals

Streptozotocin was purchased from Sigma Aldrich Chemicals Pvt, Ltd, Bangalore. All other chemicals and reagents used were of analytical grade.

2.4. Experimental induction of diabetes

All animals were allowed to adapt to cages for 3 days, after which they were fasted overnight. Diabetes was induced in rats by intra peritoneal injection of streptozotocin at a dose of 50 mg/kg, dissolved in normal saline[6]. After streptozotocin treatment, all animals were given free access to food and water. Blood glucose levels were measured 2 days after streptozotocin injection and used as parameters to match pairs of diabetic rats with similar level of severity. Only rats with fasting blood glucose levels higher than 200 mg/dL were considered to be diabetic and were used in the experiment. The mean blood concentration of glucose in normoglycemic rats was 95 mg/dL. Animals were randomly assigned to four different groups *ie*. group Ⅱ to Ⅴ. Group Ⅰ served as normal control containing 6 normal rats. All treatments started 2 days after Streptozotocin injection. Vehicles and the drugs were administered orally using oral gavage tube daily for 3 weeks. Blood samples were collected for the measurement of blood glucose level from the tail vein on 0 day, 7th, 14th and 21st day. The blood glucose level was determined by glucometer (one touch). Values of sample treated were compared with that of the standard group which was treated with glibenclamide. Then animals were sacrificed by cervical dislocation. The liver, kidney and pancreas were exposed and perfused with cold saline phosphate buffer of pH 7.4 for histopathological examination. Blood free liver and kidney were taken out and homogenized in a glass Teflon homogenizer separately (10% w/v). Incubation was done at 37 ℃ under controlled conditions for biochemical estimation. The collected blood samples were immediately centrifuged at 2 500 rpm for 15 min. The serum separated was collected in fresh serum tubes and stored in refrigerator (2-4℃) after tightly capped[7].

2.5. Antioxidant assay

2.5.1. DPPH radical scavenging activity

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH[8]. A 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of control ie. standard butylated hydroxyl toluene (BHT) at different concentration (25-100 μg/mL) and test solutions at different concentrations (5-100 μg/mL) in different test tubes. Thirty minutes later, the absorbance were measured at 517 nm.

2.5.2. Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by the spectrophotometric method[9]. Sodium nitroprusside (5 mM) in phosphate-buffer saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (5-100 μg/mL) were dissolved in methanol and incubated at 25 ℃ for 30 min. After 30 min, to 1.5 mL of the incubated solution was diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dichloride). The absorbance of the chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylene diamine dihydrochloride was measured at 546 nm.

2.5.3. Superoxide scavenging activity

Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method[9]. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. The filtrate (200 μL) was added to 2.8 mL of an aqueous solution containing nitroblue tetrazolium (56 μM), ethylenediaminetetraacetic acid (EDTA) (10 μM) and potassium phosphate buffer (10 μM, pH 7.4). Test solutions at different concentrations (5-100 μg/mL) were added and absorbances were recorded at 560 nm against the control.

2.5.4. Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was determined according to the modified method[10]. The assay was performed by adding 0.1 mL of EDTA, 0.01 mL of ferric chloride, 0.1 mL of hydrogen peroxide, 0.36 mL of deoxyribose, 1.0 mL of test solutions (5-100 μg/mL) in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid were dissolved in sequence. The mixture was then incubated at 37 ℃ for 1 hr and 1.0 mL portion of the incubated mixture was mixed with 10% TCA and 1.0 mL of 0.5% TBA to develop the pink chromogen and measured at 532 nm.

2.6. Statistical analysis

Data were statistically calculated by utilizing one way ANOVA and expressed as mean±SEM followed by Donnett's *t*-test using computerized graph pad in stat version 3.05 graph pad software USA. Differences of $P<0.05$ were considered statistically significant.

3. Results

3.1. Anti-hyperglycemic activity of Hyptis suaveolens

The effect of 50% ethanolic extract of leaves of *Hyptis suaveolens* on streptozotocin-induced animals is shown in Table 1 and Table 2. Table 1 showed the effect of oral administration of 250 mg/kg/day and 500 mg/kg/day of HSE on blood glucose level. After 21 days, at the end of treatment, reduction in blood glucose level of treated rats with dose 250 mg/kg was 116.50-219.50 mg/dL ($P< 0.01$). After oral administration of 500 mg/kg of HSE, blood glucose level in 21 days was 98.83-237.00 mg/dL($P< 0.01$). While in case of glibenclamide 600 μg/kg, it was 94.50-232.33 mg/dL ($P< 0.01$). Table 2 showed that the serum triglyceride (TG), total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were significantly higher in diabetic rats compared to those in normal and drug treated rats, while high density lipoprotein (HDL) levels were significantly decreased in the model rats compared to those in normal rats. After the treatment with 50% ethanolic extract of *Hyptis suaveolens* in the diabetic rats, a significant reduction in serum cholesterol, TG, LDL and VLDL were observed. In antioxidant assay, potential decrease in the concentration of DPPH radial due to scavenging property of methanolic extract of *Hyptis suaveolens* (MEHS) and BHT showed significant free radical scavenging activity *viz*. 86.44% and 91.86%, respectively at 100 μg/mL. The scavenging of nitric oxide by MEHS and BHT was concentration dependent. There was a moderate inhibition of nitric oxide formation with the maximum inhibition being 74.12% and 82.14%, respectively at 100 μg/mL MEHS and BHT. The MEHS and BHT showed a moderate inhibition of the superoxide radical 75.50% and 81.87%, respectively at 100 μg/mL.

The effect of MEHS and BHT on hydroxyl radical and iron (Ⅱ)-dependent deoxyribose damage was protected significantly at all concentrations. The percentage of inhibition of hydroxyl radical being 68.84% and 73.56% respectively at 100 μg/mL(Table 3).

3.2. Histopathological studies

3.2.1. Pancreas

Microscopically examined pancreas section of control group (1A) showed normal architecture with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous stroma into lobules. No fibrosis or any inflammation was seen.

Microscopically examined pancreas section of diabetic control group (1B) showed architecture with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous stroma into lobules. Focal lymphocytic infiltrate was present in stroma.

Microscopically examined pancreas section of 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group (1C and 1D) showed normal architecture with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous stroma into lobules. No fibrosis or any inflammation was seen.

The normal architecture was restored to the same as that of the standard drug (Glibenclamide) treated pancreas (1E).

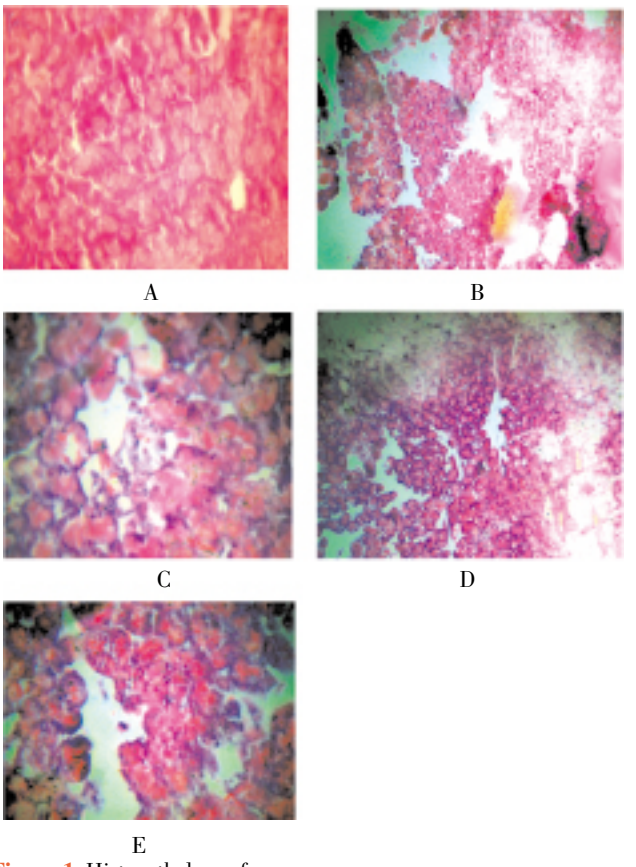

Figure 1. Histopathology of pancreas.
A: Control group; B: Diabetic control group; C: 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group at 250 mg/kg; D: 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group at 500 mg/kg; E: Glibenclamide treated group.

3.2.2. Kidney

Microscopically examined kidney section of control group (2A) showed the glomerulli appear normal. The tubules stroma appeared normal.

Microscopically examined kidney section of diabetic control group (2B) showed the moderate increase in mesengial cellularity and matrix. Tubules appeared normal and lined by single layer of cuboidal cells. The stroma revealed focal lymphoplasmacytic infiltrates.

Microscopically examined kidney section of 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group (2C) showed the moderate increase in mesengial cellularity and matrix. Tubules appeared normal. The stroma showed mild infiltration by lymphocytes, plasma cells and glomeruli and mild increase in mesengial cellularity. The tubules appeared normal. The stroma was infiltrated by lymphoplasmacytic cells (2D).

The normal architecture was restored to the same as that of the standard drug (Glibenclamide) treated kidney (2E).

3.2.3. Liver

Microscopically examined liver section of control group (3A) showed normal architecture with central veins, sinusoids and portal triads appeared normal. Hepatocytes showed moderate cytoplasm and round to oval nuclei.

Microscopically examined liver section of diabetic control group (3B) showed architecture was distorted. The central veins showed congestion. Hepatocytes showed feathery degeneration. Portal triads showed peri-portal inflammation

composed of lymphocytes.

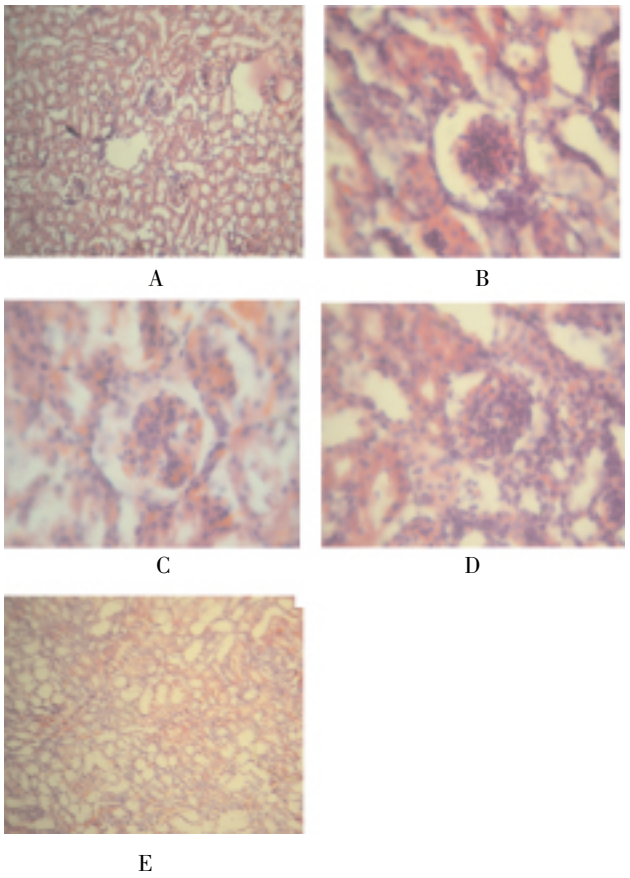

Figure 2. Histopathology of kidney.
A: Control group; B: Diabetic control group; C: 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group at 250 mg/kg; D: 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group at 500 mg/kg; E: Glibenclamide treated group.

Microscopically examined liver section of 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group (3C) showed the architecture was partially effaced. There was chronic venous congestion and patchy necrosis. The portal triads showed hepatocytes had feathery degeneration and mild chronic inflammation composed of lymphocytes. Portal triads were normal. Hepatocytes appeared normal and showed moderate cytoplasm and round to oval nuclei. There was no feathery degeneration of the hepatocytes (3D).

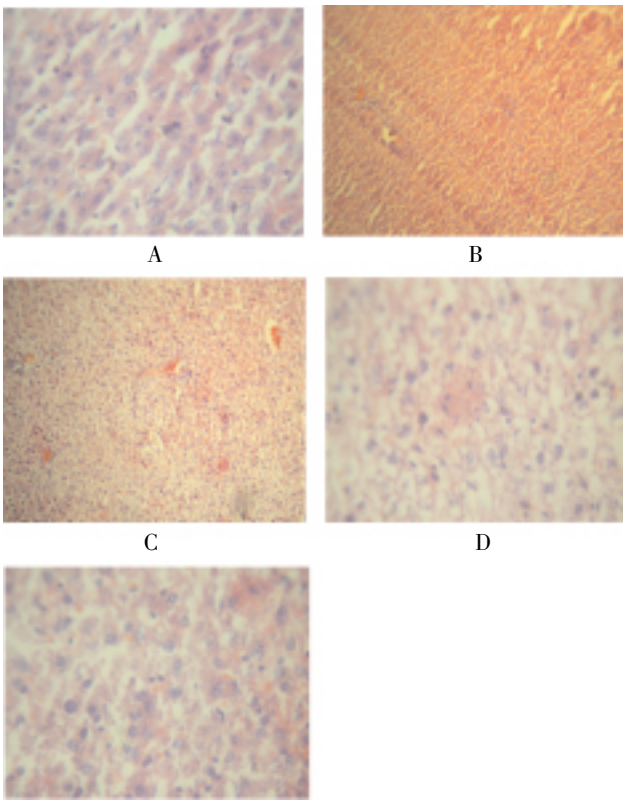

Figure 3. Histopathology of liver.
A: Control group; B: Diabetic control group; C: 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group at 250 mg/kg; D: 50% ethanolic extract of leaves of *Hyptis suaveolens* treated group at 500 mg/kg; E: Glibenclamide treated group.

Table 1
Effects of 50% ethanolic extract of leaves of *Hyptis suaveolens* on blood glucose levels in streptozotocin induced diabetic rats(Mean ± SEM)(mg/dL).

Treatment	0 day	7 days	14 days	21 days
Normal control	76.16±5.36	75.66 ± 3.94	75.00±4.96	75.00±4.61
Diabetic control	220.83±19.00	214.50± 10.60**	211.33±20.30**	208.16±17.38 **
HSE(250 mg/kg)	219.50±13.25	191.00±15.35	164.50±19.45	116.83±19.16 △△
HSE(500 mg/kg)	237.00±15.00	190.16±16.14	132.66±11.01 △	98.83±10.55 △△
Glibenclamide(600 μg/kg)	232.33±13.90	184.83±12.80	129.83±19.20△	94.50±5.46 △△

** $P<0.01$ *vs*. normal control; △$P<0.05$,△△$P<0.01$ *vs*. diabetic control.

Table 2
Effects of 50% ethanolic extract of leaves of *Hyptis suaveolens* on the total cholesterol and triglyceride level in blood serum after 21 days(Mean ± SEM)(mg/dL).

Treatment	TG	Total cholesterol	HDL	LDL	VLDL
Normal control	67.25 ± 4.54	74.36 ± 5.46	23.80 ± 2.10	35.30 ± 3.30	13.30 ± 1.70
Diabetic control	112.62 ± 6.36	152.86 ± 7.54**	13.30 ± 3.10**	71.70 ± 8.30**	30.30 ± 1.10**
HSE (250 mg/kg)	71.54 ± 5.25	78.36 ± 5.38△	18.20 ± 0.90△△	47.20 ± 6.70△	20.70 ± 2.10△
HSE (500 mg/kg)	68.32 ± 4.37	72.52 ± 6.56△△	18.90 ± 0.90△△	45.20 ± 5.30△	19.00 ± 2.00△
Glibenclamide(600 μg/kg)	82.46 ± 4.23	89.52 ± 6.46△△	19.20 ± 1.00△△	40.20 ±4.50△△	17.80 ± 2.00△△

** $P<0.01$ *vs*. normal control; △$P<0.05$,△△$P<0.01$ *vs*. diabetic control.

Table 3
Free radical scavenging activity of MEHS (Mean± SEM)(%).

Drug	Concentration (μ g/mL)	DPPH radical inhibition	Nitric oxide	Superoxide inhibition	Hydroxyl radical inhibition
MEHS	5	11.240±0.517	10.590±0.112	11.870±0.016	11.040±0.135
	10	22.360±0.453	22.720±0.451	26.690±0.592	24.580±0.876
	25	51.440±0.004	51.140±0.009	68.460±0.158	54.630±0.745
	50	72.640±0.005*	72.920±0.001*	70.430±0.364*	61.930±0.984*
	100	86.440±0.002**	74.120±0.001**	75.500±0.654**	68.840±0.647**
BHT	25	83.120±0.141	76.690±0.054	75.480±0.784	68.650±0.386
	50	86.940±0.125*	80.120±1.215*	76.020±0.887*	71.880±0.423*
	100	91.860±0.156**	82.140±0.512**	81.870±1.246**	73.560±0.368**

*P<0.05, ** P<0.01 as compared to standard.

The normal architecture was restored to the same as that of the standard drug (Glibenclamide) treated liver (3E). It was evident that 50% ethanolic extract of leaves of *Hyptis suaveolens* possessed good antidiabetic activity.

4. Discussion

Streptozotocin as an antibiotic and anticancer agent has been widely used for inducing type Ⅰ diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic β-cells[11]. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory process, which in turns leads to accumulation of lipids such as cholesterol and triglyceride in diabetic patients. The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots. In oxidative stress, where large quantities of reactive oxygen species (ROS) like hydrogen peroxide, superoxide, hydrogen radical, singlet oxygen and nitrogen species are generated, one of the earliest responses to stress. These ROS have a role in disease and aging in animals. The antioxidative system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidants such as BHT, as they are suspected to be carcinogenic. Natural antioxidants therefore have gained importance[12]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants[13]. The significant decrease in the concentration of DPPH radical is due to the scavenging ability of MEHS. Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. MEHS was shown significant scavenging activity. *Hydroxyl radicals* are the major active oxygen species that cause lipid oxidation and enormous biological damage[14]. The MEHS has potent antioxidant and free radical scavenging effects in different *in-vitro* systems. The present studies demonstrated that the 50% ethanolic extract of *Hyptis suaveolens* had an antihyperglycemic effect in the streptozotocin induced diabetic rats when administered orally along with antioxidant as well as decrease the cholesterol and triglyceride levels in the significant manner. The current study provides some useful insight into the antihyperglycemic potency of *Hyptis suaveolens* leaves in streptozotocin induced diabetes. However, we suggest that further work should be carried out at molecular level to find out the absolute mechanism of action of plant *Hyptis suaveolens* in experimental diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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