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Research Article

ANTIOXIDANT EFFECT OF *MUSA SAPIENTUM* L. (MUSACEAE) BARK JUICE

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ABSTRACT

Diabetes is a chronic disorder of metabolism of carbohydrates, proteins and fats due to absolute or relative deficiency of insulin secretion and with varying degree of insulin resistance. Oxidative stress generation occurring during chronic diabetes leads to the formation of free radicals with various complications like neurodegenerative effects. It was well reported that *Musa sapientum* bark juice possess antidiabetic as well as antioxidant activity. As the chronic diabetes and related oxidative stress were the major causes of gastroparesis, it was investigated that whether the antioxidant effect of *Musa sapientum* bark juice was effective during the treatment of gastroparesis. Gastroparesis affects various gastric functions such as gastric emptying, gastric motility, etc. which may affects the bioavailability of orally administered drugs. Hence, the impact of gastroparesis and administration of *Musa sapientum* on the plasma levels of oral antidiabetic agent (Metformin) was also studied with the help of HPLC.

Keywords: *Musa sapientum,* GOD-POD method, Antioxidant, Metformin.

INTRODUCTION

Musa sapientum L. (Musaceae) Perennial, rhizomatous, tall erect herb. Pseudo stem 2.4-4.5 m with oblong leaves 1.2-1.8 m long. Spike soon decurved and finally dropping,90 cm or more long with very large ovate deep red or dull purplish, more or less pruinose bracts, lower 15-20 cm long and deciduous, upper often forming a club. Lower bracts with numerous hermaphrodites greenish or yellowish flower about 3.8 cm long. Fruit oblong, 3-gonous in the wild form, about 7.5 cm long with very astringent, scanty, flesh and numerous black or brownish black rugose seeds[1,2]. The plant has been used in treatment of diabetes, dysentery. The roots are anthelminitic, antiscorbutic, depurative and tonic. The tender leaves are useful in scabies, inflammations, opthalmopathy, burns. The fruits are astringent, emollient, ansthelmintic useful in vitiated conditions of pitta, dipsia, scabies, and nephropathy. The flowers are good for dysentery, ascites and dropsy. The juice of bark useful in diabetes[3,4].

Growing parts of the plant contain much tannin and gallic acids. Ripe fruit contains 22% of suger, 16% being crystllizable. Besides sugar it contains starch albumunoids 4-8%, fat up to 1%, non nitrogenous extractives 6 to 13% and ash containing phosphoric anhydride, lime, alkalies, iron, chlorine etc. there are large quantities of c vitamins and a certain amount of B vitamins in it. But there is conflicts of evidence over the existence of vitamin A. Banana is rich in vitamins capable of preventing and curing diseases due to vitamin A deficiency, and that to a less extent, or at any rate more slowly, the vitamins in the banana promote growth. juice of the bark of plantain contains, potash, soda, lime, magnesia, gallocatachin. Juice of the tender roots contains much of tannin[5-8].

The juice of the bark and leaf is frequently given to children suffering from an overdose of opium. The juice of bark mixed with acts as a brisk purgative, also used in diabetes. Young plantain leaves are used as cool dressing for blisters, burns and to retain the moisture of water dressings. They may also be used as a green shade in ophthalmia and other eye diseases[1,6-8].

MATERIALS AND METHORDS

Collection of *Musa Sapientum* Bark Juice

Fresh *Musa sapientum* L. bark juice was collected from local area.

Animals

Having obtained the approval of the Institutional Animal Ethics Committee, albino Wister rats of both sex and average weight (200-220 g) were housed at controlled temperature (25± 2°C) with food and water *ad libitum*. They were fasted for 18 h for before experimentation. Optimum care was exercised to avoid coprophagia.

Chemical and Reagents

The test drug Metformin, Alloxan monohydrate, Trichloroacetic acid (TCA), Phenol red, Topfer's reagent, Methylcellulose and Acetyl choline.

Induction of Diabetes

The diabetes was induced experimentally in rats by the intraperitoneal administration of 120 mg/kg of alloxan freshly prepared in saline followed by intermittent dose of 100 mg/kg on 12th and 21st day. The rats were given 5% glucose *ad libitum* for 24 hrs in order to prevent transient hypoglycemia[9,10].

Assessment of Diabetes

The blood glucose levels were determined after 72 hrs, 15th and 43rd day after the administration of alloxan. The blood was collected by micro-capillary technique from the retro-orbital plexus under light ether anesthesia and was used for blood glucose estimation. The rats showing blood glucose more than 172 mg/dl were considered diabetic[11].

Assessment of Gastroparesis

The gastroparesis was assessed by various gastric parameters such as Gastric emptying: [10, 12]

The gastric emptying of a non-nutrient solution was assessed by previously reported method. After the above-mentioned treatments, on day 43, the rats received a 1.5 ml test meal consisting of 0.05% phenol red in 1.5% aqueous methylcellulose solution by intragastric route. The animals were sacrificed after thirty minutes. The stomach was excised and homogenized along with its contents in 20.0 ml of 0.1 N NaOH. In 5.0 ml of stomach homogenate, trichloroacetic acid (0.2 ml of 20% w/v) was added to precipitate proteins and centrifuged. The supernatant was mixed with 4.0 ml of 0.5 N NaOH and read spectrophotometrically at 560 nm. Phenol red recovered from the stomach of rat sacrificed immediately after administration of methylcellulose meal served as standard stomach. The percentage gastric emptying of each rat was calculated from the following formula:

$$\% \text{ Gastric emptying} = 1 - \frac{\text{Absorbance of the solution from test stomach}}{\text{Average absorbacne of the solution from standard stomach}} \times 100$$

Contractility of Fundus and Pylorus[13,14]

The stomach of overnight fasted rat was incised and the fundus and pylorus were isolated. Isolated tissues were washed with fresh aerated Krebs solution. Both the fundus and pylorus were cut in a zigzag manner and were set in a specially designed organ bath assembly under standard experimental conditions. After stabilization for 30 min, the different concentrations of acetylcholine

(Ach) were tested and the minimum concentration giving maximum contractile response of the tissue was identified. This was considered as 100% response. The graph for log dose vs. percent response was plotted and EC50 values for ACH were determined for each tested tissue from each treated group of rats.

Gastric Acid Secretion[13]

The rats were sacrificed with deep ether anesthesia. The abdomen was opened and pylorus was ligated. The stomachs were removed and the content of stomach was centrifuged and volume of supernatant fluid was measured. 0.1 ml of the same was titrated against 0.01 N NaOH using Topfer's as an indicator. The total gastric acid secretion is expressed as total acid output (µEq/100 g body weight).

Determination of Plasma Level of Metformin

The metformin was administered intraperitonially. After the drug administration the blood from three rats of each group was collected for each time intervals. The plasma level of metformin was determined by HPLC method reported earlier[15].

Chemical and Reagents

Metformin, Acetonitrile (HPLC grade) and ammonium acetate (AR grade), Nylon 6, 6 Membrane filters (0.2 µm, 47 mm) and Nylon 6, 6 Membrane filters (0.2 µm, 25 mm).

Chromatography

Systronic HPLC system consisted of a model LC 6600 pump, and model C 2000 U.V. visible detector. The separation was performed on an analytical 250 X4.6 mm (5um partical size).Eurospere-100 C_{18} column. The wavelength was set at 236 nm. The mobile phase was 10% Acetonitrile, 90% 0.15M Ammonium Acetate adjusted to pH 6.5 at flow rate 1 ml/min. The mobile phase was prepared Daily and degassed by ultrasonication before use. The mobile phase was not allowed to recirculate during the analysis.

Plasma Sample Treatment

To 0.5 ml of plasma, in a 1.5 ml Centrifuge tube, 0.5 ml acetonitrile was added to precipitate proteins. Further 0.5 ml mobile phase was added. Centrifuge tubes were vortex-mixed for 30s to bring about complete extraction from proteins and after standing for 10 min; the tubes were centrifuged at 5000 rpm for 10 min. The upper layer (about 100 µl) was separated with the help of syringe and filtered through 0.2 µm, 25 mm Nylon membrane filters. About 20 µl of filtrate was injected onto the HPLC system.

Standard Solutions

Stock solutions (1mg/ml) and appropriate dilutions of metformin and were prepared in methanol and stored at +4 ^{0}C. No change in stability over the period of 2 weeks was observed.

Sample Preparation

To 100 µl of plasma in a glass stoppered 10 ml Centrifuge tube were added 10 µl of metformin (1 µg/ml). After mixing (30 s) the mixture centrifuge for 15 min at 6000 rpm. Then 20 µl of supernatant was injected onto the HPLC system.

Biological Samples

Metformin was administered in a single dose of 500 mg/kg to albino Wistar rats after 1 h plasma sample were collected and frozen immediately at 20^{0}C until assayed.

RESULT AND DISCUSSION

The main objective of the present study whether the antioxidant effect of *Musa sapientum* bark juice was effective during the treatment of gastroparesis and to study its impact on bioavailability of oral antidiabetic agent (Metformin).

The present investigations thus necessitated a need of animal model in which hyperglycemia can exist for at least 6-8 weeks so that it can produce detectable pathological condition in GIT.. Taking these points in consideration the present study employed multiple doses of alloxan (120 mg/kg on 1st day followed by 100 mg/kg on 12th and 21st day) so as to prevent reversion of hyperglycemia.

The results shown in Fig. 1 revealed the effect of alloxan treatment on blood glucose levels of the rats on day 3rd, 12th and 43rd day. It was seen that there was significant increase in the blood glucose levels after alloxan treatment.

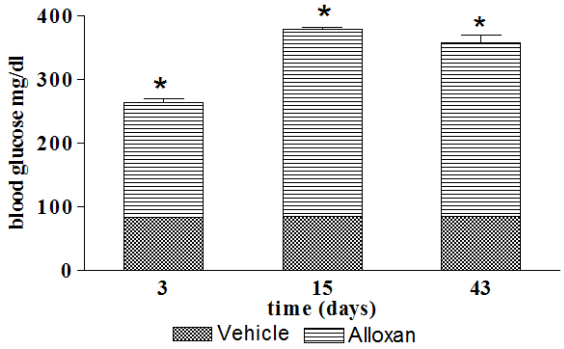


Fig. 1: Effect of alloxan treatment on blood glucose levels.

Results are expressed as mean±SEM (n=5)

*P<0.001, when compared to vehicle treated group.

Chronic diabetes generates oxidative stress leading to neurodegenerative disorders which includes gastrointestinal disorder like gastroparesis. Gastroparesis is delayed gastric emptying in which stomach takes too time to empty its content. Gastric emptying is passage of stomach content from stomach to the small intestine. Along with the delayed gastric emptying, decreased gastric contractility and decreased gastric secretion were also the symptoms of the gastroparesis.

Fig. 2 shows the effect of Musa sapientum bark juice on gastric emptying time in chronic diabetic rats. It was observed that there was a significant increase in gastric emptying time in the rats of diabetic group as compared to control group. Treatment with Musa sapientum juice significantly decreases gastric emptying time near to normal level.

Fig. 3-4 shows the effect of *Musa sapientum* bark juice on fundus and pylorus contractility in chronic diabetic rats. It was observed that there was significant decrease in fundus and pylorus contractility in the rats of diabetic group as compared to control group. Treatment with *Musa sapientum* juice significantly attenuated the above effect on the contractility of fundus and pylorus.

Thus, the observed impaired contractibility of gastric tissues in diabetic condition is probably responsible for the observed delayed gastric emptying in diabetic group.

Fig. 5 shows the effect of *Musa sapientum* bark juice on gastric acid secretion in chronic diabetic rats. It was observed that there was a significant decrease in gastric acid secretion in the diabetic rats when compared to control rats. This effect was attenuated with the treatment of *Musa sapientum* juice by increasing the gastric acid secretion significantly near to normal level.

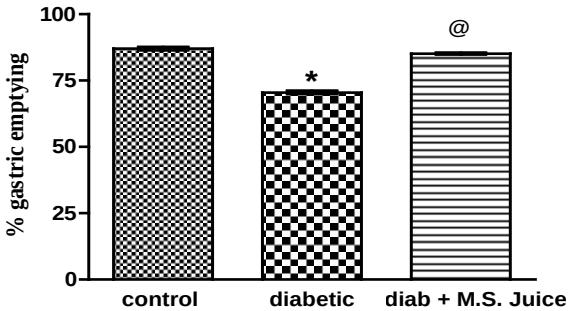


Fig. 2: Effect of diabetes on gastric emptying.

Results are expressed as mean±SEM (n=6)

*P<0.001 when compared to control group and @P<0.01 when compared to diabetic group.

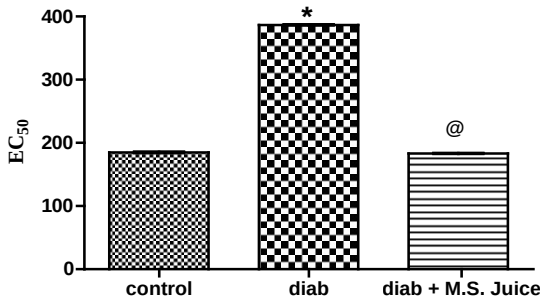


Fig. 3: Effect of diabetes on fundus contractility.

Results are expressed as mean±SEM (n=6)

*P<0.001 when compared to control group and @P<0.01 when compared to diabetic group.

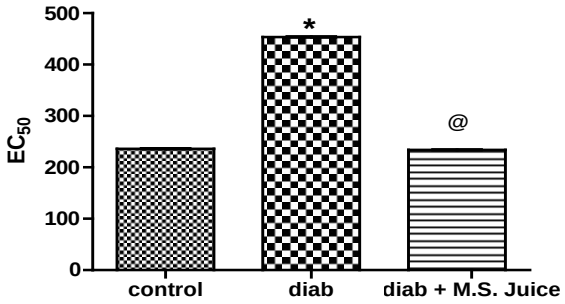


Fig. 4: Effect of diabetes on pylorus contractility.

Results are expressed as mean±SEM (n=6)

*P<0.001 when compared to control group and @P<0.01 when compared to diabetic group.

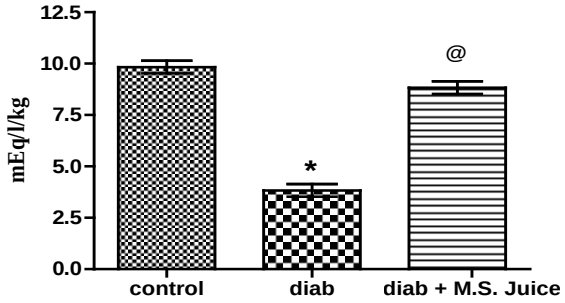


Fig 5: Effect of diabetes on gastric secretion.

Results are expressed as mean±SEM (n=6)

*P<0.001 when compared to control group and @P<0.01 when compared to diabetic group.

Determination of Plasma Level of Metformin

Investigations carried out they indicated that experimental induction of diabetes for six weeks produced detectable and significant impairment in gastric functions of rats.

Further, it was observed that treatment with *Musa sapientum* juice significantly attenuated hyperglycemia-induced changes in gastric functions, Gastric emptying time, GI motility and gastric pH are some of the important determinants that affects the absorption kinetics of any orally administered drug which onward reflect on its ultimate plasma levels. Clinically, it is often evident that the diabetic patients having disturbed GI function exhibit poor hyperglycemic control with the oral antidiabetic agents.

In view of these evidences, it was speculated that the GI dysfunction produced by hyperglycemia via oxidative stress may reflect upon the plasma levels of oral antidiabetic agents. In order to find the effect of *Musa sapientum* on the plasma levels of metformin was ere studied in diabetic rats after their oral administration.

The estimation of plasma levels of metformin was carried out by earlier reported HPLC methods. Since frequent withdrawal of blood for estimating the plasma levels at various time intervals was not possible in the same animal, the *sparese* sampling technique was employed for which a group of three animals for each time interval.

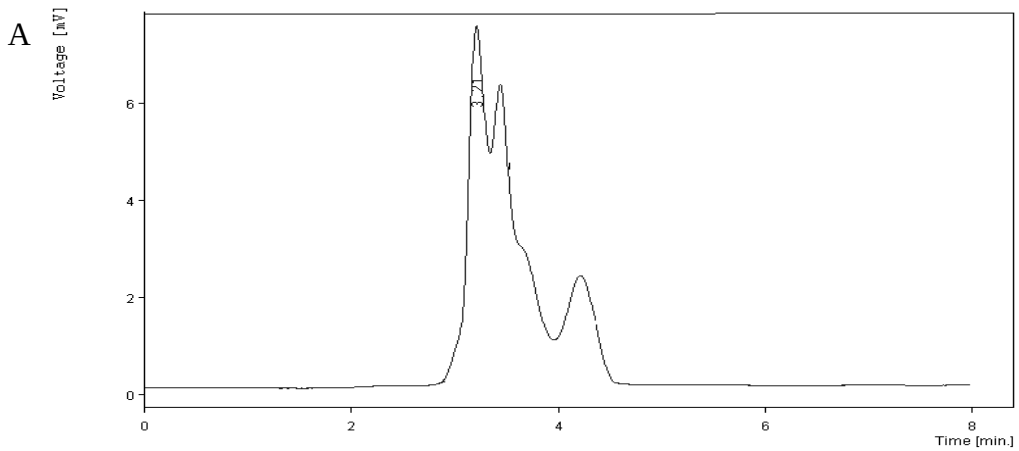
The chromatographic analysis reveled that metformin peak was distinctly detected at 4.72 min. The metformin was detected at 236 nm with UV-VIS detector on C-18 column (Bond pack).

Fig. 6A, 6B and 6C show the peaks for plain plasma, plasma spiked with metformin and plasma sample at 1hr respectively and indicate that there was no substantial plasma peak interference with metformin peak.

In vivo plasma level time study of metformin demonstrated (Fig. 7) that C_{max}, AUC were significantly decreased in case of diabetic animals when compared with the control group along with significant increase in T_{max} (Fig. 4). The other absorption kinetic parameters such as V_d and constant of absorption (K_{abs}) for metformin were significantly reduced in case of diabetic group when compared with the control group (Fig. 4). All of these parameters were significantly attenuated with the treatment of *Musa sapientum* (Fig. 4).

The observed decrease in plasma levels of test drugs due to reduced absorption in diabetes state appears related to GI dysfunctions as the same has been significantly improved by *Musa sapientum* treatment.

In view of these evidences it appears that the low levels of metformin exhibited by diabetic group is consequent to the hyperglycemia led changes in GI functions.



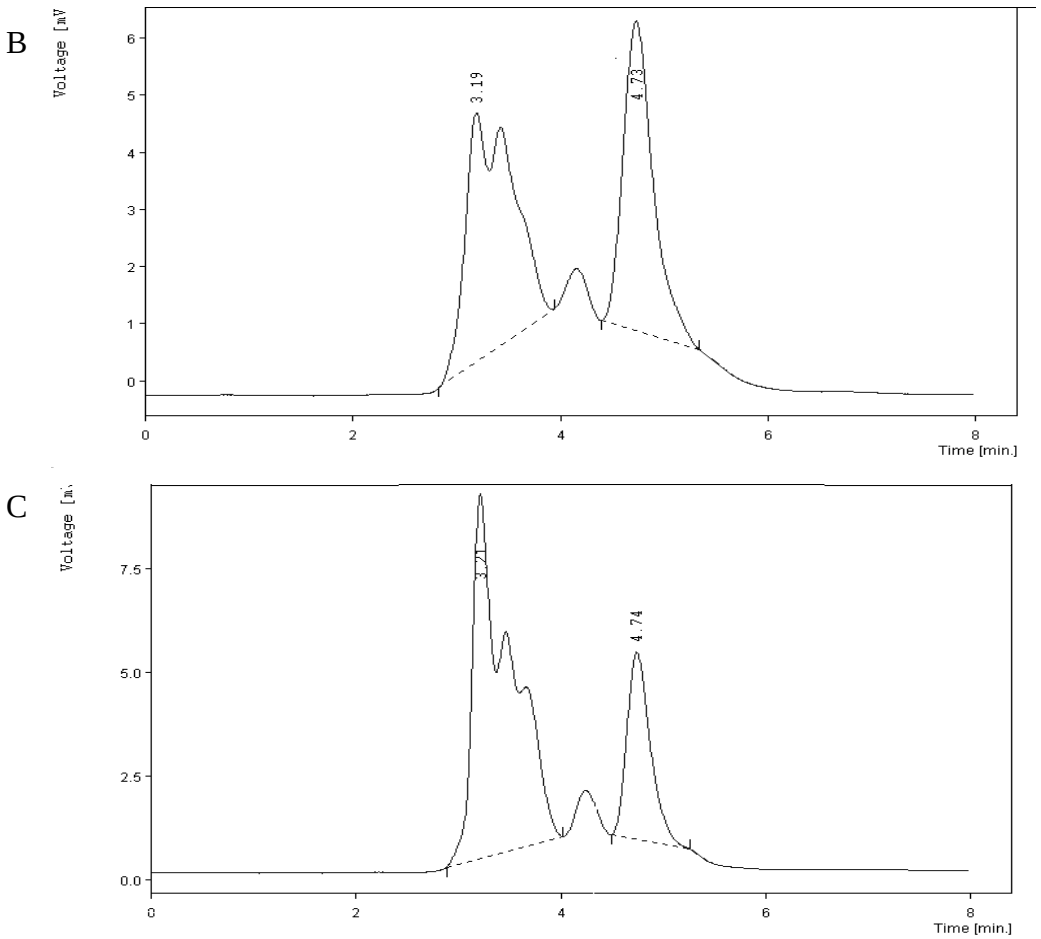


Fig. 6: Typical HPLC Chromatograms of Metformin.

A. Blank Serum Chromatogram, **B**. Chromatogram of rat plasma spiked with Metformin, **C.** Chromatogram of rat plasma sample at 1 h for Metformin.

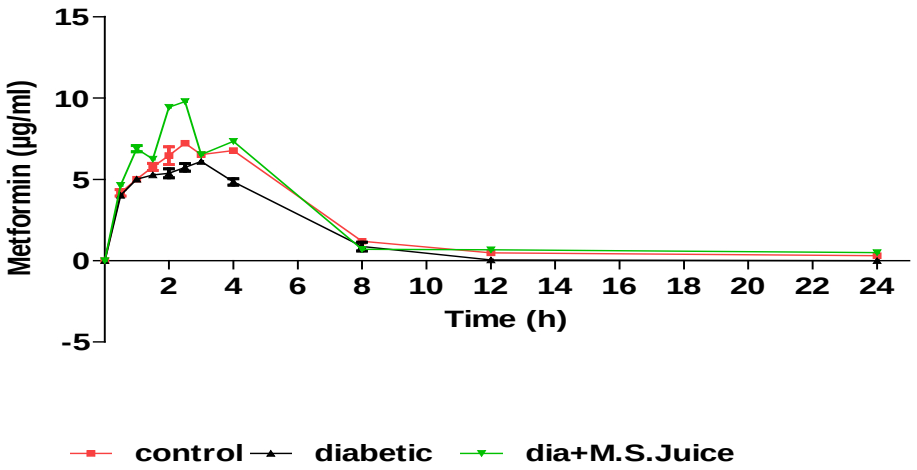


Fig. 7: Effect of diabetes induced gastric dysfunction on plasma Metformin levels.

CONCLUSION

In present study, *Musa Sapientum* bark juice was collected from local area. Diabetes was induced in rats by administering alloxan saline solution and mentioned for six week. Assessment of diabetes was seen by GOD-POD method. Oxidative stress generation occurring during chronic diabetes leads to the formation of free radicals with various complications like neurodegenerative effects. Due to neurodegenerative effect vagus which controls the movement of the digestive tract was damaged. The damage to the vagus nerve, the muscles of the stomach and intestines do not work normally and the movement is slowed or stopped resulting in generation of gastroparesis.

Assessment of gastroparesis was done by assessing various parameters like gastric emptying, fundus and pylorus contractility and gastric acid secretion. The common cause gastroparesis is diabetic induced oxidative stress. In oxidative stress there is excess

formation and insufficient removal of highly reactive molecules such as reactive oxygen species and reactive nitrogen species. Reactive oxygen species include free radicals. It was well reported that *Musa sapientum* bark juice possess antidiabetic as well as antioxidant activity. As the chronic diabetes and related oxidative stress were the major causes of gastroparesis, it was investigated that whether the antioxidant effect of *Musa sapientum* bark juice was effective during the treatment of gastroparesis.

Gastroparesis affects various gastric functions such as gastric emptying, gastric motility, etc. which may affects the bioavailability of orally administered drugs. Hence, the impact of gastroparesis and administration of *Musa sapientum* on the plasma levels of oral antidiabetic agent (Metformin) was also studied with the help of HPLC.

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