

Full Length Research Paper

Comparative study between the effect of *Coccinia cordifolia* (leaf and root) powder on hypoglycemic and hypolipidemic activity of alloxan induced type 2 diabetic Long- Evans rats

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The aim of this study is to investigate the hypoglycemic and hypolipidemic effects of *Coccinia cordifolia* leaf and root powder on alloxan induced type 2 diabetic Long-Evans rats. Oral feeding of the *C. cordifolia* leaf and root powder slightly decreased serum total cholesterol, triglyceride levels and LDL-cholesterol as compared with leaf, root and standard drug. *C. cordifolia* leaf showed more significant ($p < 0.05$, 0.01 and 0.001) effect on blood glucose level when compared with root and standard drug (glibenclamide, 5 mg/kg). At the mean time, rats' serum insulin level markedly increased; leaf does showed more significant ($p < 0.05$, 0.01 and 0.001) effect than glibenclamide control group and root powder feeding group. But *C. cordifolia* did not show any significant effect on HDL-cholesterol and liver glycogen after 21st day feeding. Thus, the results of the experimental study suggest that *C. cordifolia* possesses hypoglycemic and hypolipidemic effects and is able to ameliorate the diabetic state and can be served as a source of potent antidiabetic agent. In this comparative study, it was shown that leaf was better than root in the effects of hypoglycemic and hypolipidemic status.

Key words: Type 2 diabetes mellitus, *Coccinia cordifolia*, alloxan, hypoglycemic, hypolipidemic, LDL-cholesterol and glibenclamide.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system (Bhagwat, 2006). Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus (Tiwari, 2005). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra et al., 2006). In one study, it was shown that about two third of the total diabetic patients by the year 2030 would be constituted from

those of the developing countries particularly, China, India, Indonesia, Pakistan and Bangladesh (Wild et al., 2004). So, worldwide surveillance of diabetes is a necessary first step toward its prevention and control, which is now recognized as an urgent priority (King et al., 1998). Plants are well known in traditional herbal medicine for their hypoglycemic activities, and available literature indicate that there are more than 800 plant species showing hypoglycemic activity (Rajagopal et al., 2008). Despite the presence of known anti-diabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic and anti-

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hyperlipidemic remedies (Shukla et al., 2000; Mahomed et al., 2003 and Hou et al., 2005).

Anti-hyperglycemic effects of various plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes (Ali et al., 2009). Ivy gourd or *C. cordifolia* is an aggressive vine in the Cucurbitaceae (cucumber) family (Linney, 2004). It has been classified as one of the medicinal herbs in the traditional practice of the Bangladeshi as well as Indian medicine. The juice of the roots and leaves is used to treat diabetes while the leaves are also used as poultice to treat skin eruptions. In addition, the aqueous and ethanolic extracts of the plant have shown hypoglycemic action (Chopra, 1986). Another study has shown improvement in glucose tolerance of *C. cordifolia* in patients with maturity onset diabetes (Azad et al., 1979). *C. cordifolia* possesses hypoglycemic and hypolipidemic effects and is able to get better the diabetic state and can be served as a source of potent anti-diabetic agent. My aim is to find out the potent compound, which is more effective on glycemetic activity.

MATERIALS AND METHODS

The leaves and roots of *C. cordifolia* were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR), Rayerbazar, Dhaka, Bangladesh, and Golapnagar, a village of Kushtia, Bangladesh. The plant was identified by the Bangladesh National Herbarium, Dhaka.

Preparation of *C. cordifolia* (leaf and root) powder

The mature fresh leaves and roots were washed carefully with water. After that, roots were cut into small pieces. The roots and leafs were air dried and then oven dried at 37°C temperature. The dried leaves and roots were then grinded to make powder, which were then screened to get fine powder.

Model animals

Healthy female rats (Long- Evans) of local strain, weighing 145 to 185 g were used in these experiments. Before using the rat for experiment, they were kept under observation for a week and maintained at a constant room temperature of $22 \pm 5^\circ\text{C}$ with humidity of 40 to 70% and the natural 12 h day-night cycle, in animal house of Animal Research Section, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. A total number of 30 rats were used to carry out the experiments, which include only type 2 diabetic model rats. Each type of experimental models was divided into five groups. Each individual group contained 5 rats, matched with body weight. These include:

- 1) Group-A: fed powder of *C. cordifolia* leaf 50% + 50% normal rat diet (wild 50%).
- 2) Group-B: fed powder of *C. cordifolia* leaf 30% + 70% normal rat

diet (wild 30%).

3) Group-C: fed powder of *C. cordifolia* root 50% + 50% normal rat diet (hybrid 50%).

4) Group-D: fed powder of *C. cordifolia* root 30% + 70% normal rat diet (hybrid 30%).

5) Group-E (fed with glibenclamide)

6) Group-F (fed normal rat diet)

Preparation of diabetic rats

The fasted rats were made diabetic by injecting alloxan monohydrate 150 mg/Kg-b.w. (dissolved in sterile normal saline, NaCl 0.9%) intraperitoneal injection (Akhtar et al., 1981). These doses disabled the β cells of pancreas to produce insulin hormone. After three days injection of the alloxan monohydrate, blood glucose increased dramatically in all of the surviving rats and was determined by the Diagnostics Elitech method. Rats with more than 6 mMol/L blood glucose levels were considered as diabetic and employed for further study.

Dose and route of administration

For the evaluation of the hypoglycemic activity, the powder of the *C. cordifolia* (leaf and root) was administered orally at a dose of 32.19 g/kg (50% sample + 50% normal diet) and 19.31g/kg (30% sample + 70% normal diet) body weight for 21 days. For all the pharmacological studies, the drug glibenclamide was administered as a positive control, at a dose of 5 mg/10 mL (9.9 mL H₂O + 0.1 ml Twin 20)/kg body weight for Type 2 model rats.

Collection of blood sample

Fasting blood samples were collected on the 3rd day (initial), 8th, 15th and 22nd by amputation of the tail tip under diethyl ether anesthesia. Just before cutting the tail, it was immersed in warm water (40°C) for approximately 22 s for vasodilatation. The level of blood glucose was determined on the 3rd (initial), 8th, 15th and 22nd day sample. In the case of the chronic study, blood was collected on the final day that is, 22nd day by sacrificing the rats. After cutting the tail tip, about 0.2 mL blood was taken cautiously, and the blood glucose level was measured by glucometer (aqua check). The 22nd day collected blood were centrifuged after 20 min at 4000 rpm for 10 min and re-centrifuged at 2000 rpm for 5 min. The serums were separated and taken into eppendorfs. Fasting on 21st day glucose, serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured. 100 μL of serum were aliquot and kept frozen at -20°C until analysis of fasting serum insulin was carried out.

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 14 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean \pm SD as appropriate. Statistical analysis of the results was performed by using the student's t-test (paired and unpaired) or ANOVA (analysis of variance) followed by Bonferroni post hoc test.

The limit of significance was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS AND DISCUSSION

The present study was undertaken to assess chronic "Comparative study between the effect of *C. cordifolia* (leaf and root) on hypoglycemic and hypolipidemic activity of alloxan induced type 2 diabetic Long-Evans rats". An attempt was also made to assess the mode of anti-diabetic action of *C. cordifolia* (leaf and root). On the 22nd day (after 21 day chronic feeding) when the 4 times glucose test was performed, powder of *C. cordifolia* (leaf and root) both showed significant anti-hyperglycemic effect in Type 2 model rats but leaf showed more significant effect. In the chronic study, the most important finding was that, after 21 days of consecutive feeding, the rats were sacrificed on the 22nd day, a significant reduction ($p < 0.05$) in the fasting glucose level was observed in leaf powder feeding group as compared with the normal control, root feeding group which is shown in Table 1 and Figure 1. In this experiment, glibenclamide and leaf (50%) treated group significantly decreased ($p < 0.01$) fasting blood glucose level after chronic feeding. But in this study, leaf (50%) showed the most significant effect.

Serum cholesterol level was also decreased among all the groups but more significant levels ($p < 0.05$, 0.01, 0.001) was found in both leaves (50%), leaf (30%) treated group and glibenclamide treated groups. The decreasing tendency of cholesterol was by 13, 08 and 13% respectively, after 21 days (on 22nd day). In these cases, leaf was more effective than root which is shown in Figure 2.

Serum triglyceride level was also decreased among all the groups but more significant levels ($p < 0.05$, 0.01, 0.001) was found in the cases of leaf (50%), glibenclamide, and leaf (30%) treated groups. The data clearly showed that the decreasing tendency of triglyceride was by 25, 21 and 15% respectively, after 21 days (on 22nd day) which is shown in Figure 3. Apart from the blood sugar lowering effect, beneficial changes in lipid profile have also been observed by powder of *C. cordifolia* (leaf and root). In this study, powder of *C. cordifolia* (leaf and root) showed significant effect ($p < 0.05$, 0.01, 0.001) on the effect of LDL-cholesterol as compared with the powder of leaf and root which showed that leaf (50%) had most significant ($p < 0.01$) effect as shown in Figure 4. So powder of leaf is more effective on the LDL-cholesterol than root. There were no significant changes in case of HDL-cholesterol level among all the test groups after 21 days of chronic experiment which is shown in Figure 5. The chronic effects of *C. cordifolia* powder (leaf and root) on the insulinemic status of type 2 diabetic model rats have been found. Serum insulin level changes significantly ($p < 0.05$) increasing in both leaves (50%), leaf (30%), and glibenclamide after 21 days of

chronic feeding. Leaf (50%), leaf (30%), and glibenclamide increased the serum insulin level 15, 16 and 15% respectively, as shown in Table 2 and Figure 6. So compared with leaf and root, leaf is more effective than root to the effect on serum insulin level.

Diabetes is a major degenerative disease in the world today (Ogbonnia et al., 2008), affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (Edem, 2009).

This present study was undertaken to compare the hypoglycemic and hypolipidemic activity of *C. cordifolia* (leaf and root). In the present investigation, it was found that in type 2 diabetic model rats alloxan produce significant increase in fasting blood glucose concentration on the 3rd day to inject alloxan the concentration was ranging between 12.92 ± 1.71 and 14.84 ± 0.30 mMol/L. Alloxan is a toxic glucose analogue, which selectively disable insulin-producing cells (β -cell) in the pancreas to produce insulin (Grover et al., 2000).

Daily feeding of *C. cordifolia* (leaf and root) powder for 21 days resulted in decreasing in the blood glucose levels of alloxan-induced diabetic rats. The leaf 50% significantly ($p < 0.05$) decreased glucose level 67% ($M \pm SD$ -14.84 to 4.88 mMol/L) after 21 days. Whereas leaf 30%, root 50%, root 30% and glibenclamide decreased 58, 51, 41 and 61% respectively. The effect of leaf 50% is more significant than the standard drug (glibenclamide) for diabetic subject. The possible mechanism by which powder brings about its hypoglycemic activity may be the potentiating of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing β -cells or by its release from the bound insulin (Shirwaikar et al., 2006).

Hyperlipid profile occurred by the induction of alloxan to the model rats. The hyper cholesterol was not significantly ($p < 0.05$) decreased but hypertriglyceride concentration significantly ($p < 0.05$) decreased 25% as compared with another group after 21 days of orally feeding of *C. cordifolia* leaf powder. The HDL-Cholesterol and LDL-Cholesterol did not significantly ($p < 0.05$) decrease or increase after 21 days. In the absence of insulin, protein production was not favored (Herfindal et al., 2001; Soyal et al., 2007). Treatment with powder has shown a significant decrease in the serum cholesterol and serum triglycerides indicating an increase in insulin level.

The present study showed a significant ($p < 0.05$, 0.01 and 0.001) increase of serum insulin level after 21 days feeding. The increase in insulin, which results in activation of glycogen synthetase system, may be due to improvement of glycogenesis. *C. cordifolia* leaf 50% powder may prevent other areas like the ongoing β -cells damage, recovery of partially damaged β -cells, and regeneration of new cells and stimulation of insulin secretion and also significantly lower the blood glucose level (hypoglycemic activity) and decrease lipid level (hypolipidemic activity).

Table 1. Chronic effect of *Coccinia cordifolia* powder (leaf and root) on blood glucose level (BGL) of type 2 diabetic model rats.

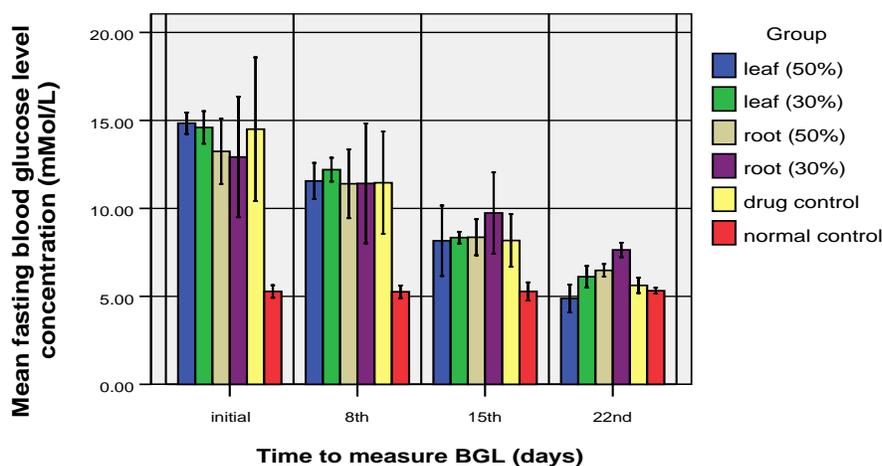
Group	Glu_3 rd day (mMol/L)	Glu_8 th day (mMol/L)	Glu_15 th day (mMol/L)	Glu_22 nd day (mMol/L)
Leaf (50%)(n=5)	14.84±0.30	11.56±.51	8.16±1	4.88±0.39 ^{abc}
Leaf (30%)(n=5)	14.6±0.43	12.2±.34	8.34±.16	6.12±0.3
Root (50%)(n=5)	13.2±0.92	11.4±0.97	8.36±.51	6.48±0.18
Root (30%)(n=5)	12.92±1.71	11.42±1.70	9.74±1.15	7.64±0.20736
Glibenclamide (n=5)	14.5±2.04	11.46±1.45	8.18±0.746	5.62±0.216 ^{abc}
Normal control (n=5)	5.28±0.18	5.26±0.18 ^{abc}	5.28±0.26 ^{abc}	5.32±0.083 ^{abc}

Data are presented as Mean ± SD; SD = standard deviation. Among groups, comparison was done using one way ANOVA with post hoc Bonferroni P test. BGC= Blood glucose Concentration; Glu = Glucose; n= number of rats. Significant level ^aP <0.05, ^bP <0.01 and ^cP <0.001 vs. root (50%).

Table 2. Chronic effect of *Coccinia cordifolia* powder (leaf and root) on Serum insulin of type 2 diabetic model rats.

Group	Insulin 3 rd (initial) day (ng/mL)	Insulin 22 nd day (ng/mL)
Leaf (50%) (n=5)	1.026±0.24	1.18±0.14 ^a
Leaf (30%) (n=5)	0.908±0.117	1.06±.110
Root (50%) (n=5)	1.04±0.12	1.09±0.15
Root (30%) (n=5)	1.08±0.14	1.1±0.09
Glibenclamide (n=5)	1.25±0.13	1.44±0.11 ^{abc}
Normal control (n=5)	1.48±0.168	1.52±0.19 ^{abc}

Data are presented as Mean ± SD; SD= standard deviation. Among groups, comparison was done using one way ANOVA with post hoc Bonferroni P test; n= number of rats. Significant level ^aP <0.05, ^bP<0.01 and ^cP<0.001 vs. root (50%).

**Figure 1.** Chronic effect of *Coccinia cordifolia* powder (leaf and root) on fasting blood glucose level (BGL) of type 2 diabetic model rats.

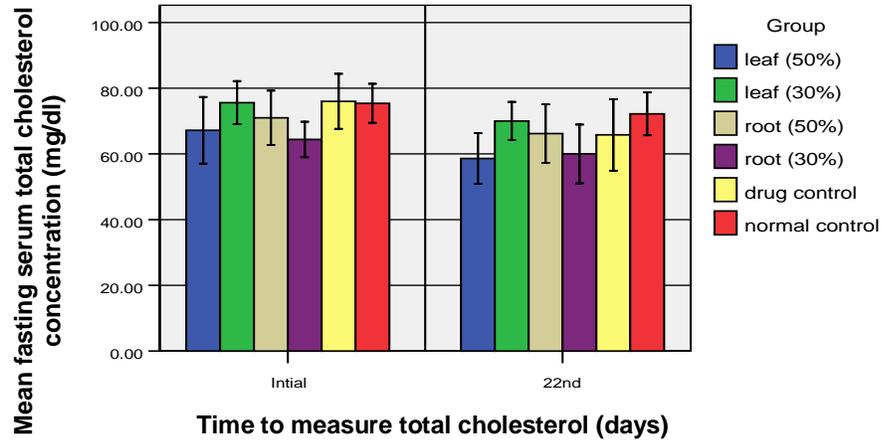


Figure 2. Chronic effect of *C. cordifolia* powder (leaf and root) on fasting serum total cholesterol concentration of type 2 diabetic model rats.

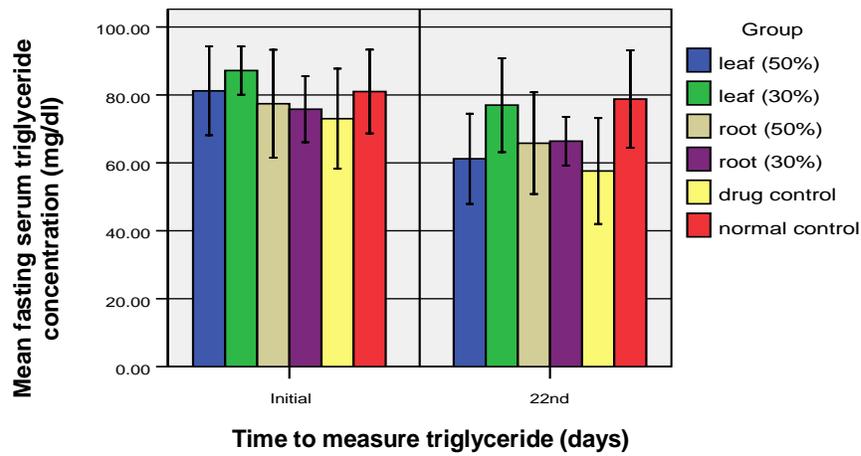


Figure 3. Chronic effect of *C. cordifolia* powder (leaf and root) on fasting serum triglyceride concentration of type 2 diabetic model rats.

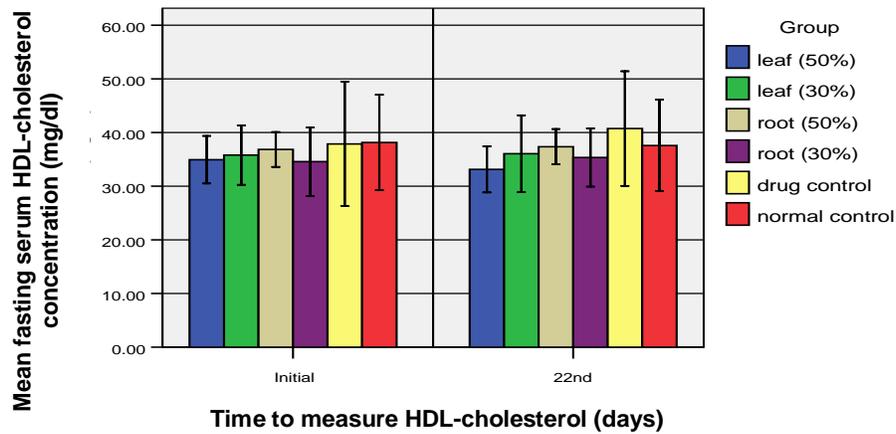


Figure 4. Chronic effect of *Coccinia cordifolia* powder (leaf and root) on fasting serum HDL- concentration of type 2 diabetic model rats.

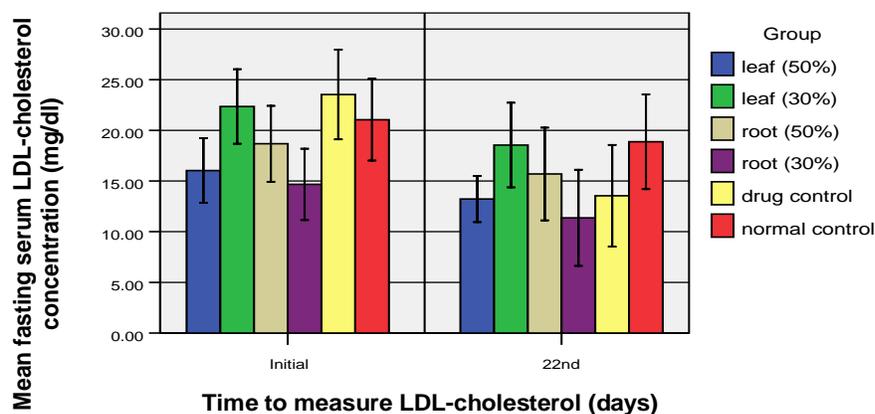


Figure 5. Chronic effect of *C. cordifolia* powder (leaf and root) on fasting serum LDL-cholesterol concentration of type 2 diabetic model rats.

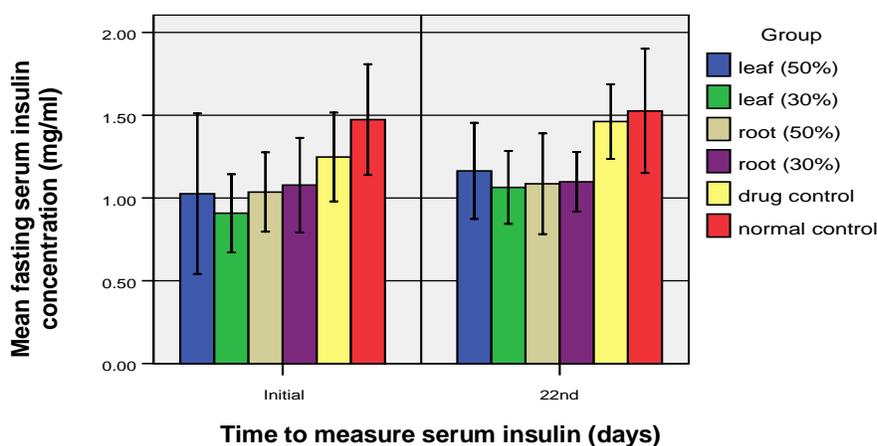


Figure 6. Chronic effect of *Coccinia cordifolia* powder (leaf and root) on fasting serum insulin concentration of type 2 diabetic model rats.

Conclusion

From this experimental finding, it is possible to conclude that powder of *C. cordifolia* (leaf and root) exhibited promising hypoglycemic and hypolipidemic activities in alloxan induced Type 2 diabetic Long-Evans rats. Hence, *C. cordifolia* can be helpful in the management of DM and other associated complications. In this study, it was stated that leaf has more activity than root in the effect of hypoglycemic and hypolipidemic status. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

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