

Antioxidant activity and free radical-scavenging of cape gooseberry (*Physalis peruviana* L.) in hepatocellular carcinoma rats model

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ABSTRACT

Aim: Oxidative damage of cellular components by free radicals and other reactive oxygen molecules is believed to be associated with the development of degenerative diseases. The aim of the present study was to evaluate the antioxidant capacity and free radical scavenging activity of cape gooseberry juice (CGJ) in diethylnitrosamine-(DENA) and CCl₄ (3 mL/kg b.w.)-induced hepatocellular carcinoma (HCC) rats model. **Methods:** The rats were divided into 4 groups (6 rats each group). Group 1 (control): the rats of this group did not receive any treatments; group 2 (CGJ): rats were daily administered cape gooseberry juice at a dose of 1 mL/kg b.w.; group 3 (HCC): the rats treated with a single intraperitoneal injection of fresh DENA (200 mg/kg body weight) and received a subcutaneous injection of CCl₄ (3 mL/kg/week); group 4: (HCC + CGJ): rats were treated with DENA (200 mg/kg b.w.) and CCl₄ (3 mL/kg b.w. per week) in addition to daily administered cape gooseberry juice at a dose of 1 mL/kg b.w. **Results:** Treatment



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with DENA plus CCl₄ induced a significant increase in tumor marker level, alpha-fetoprotein level, and liver function enzymes activity as well as elevated levels of malondialdehyde. This suggests oxidative stress accompanied with a significant decrease in antioxidant biomarkers including glutathione, total antioxidant capacity, superoxide dismutase and catalase in the examined tissues. However, the administration of GGJ could reduce these changes to control levels. **Conclusion:** CGJ is a promising candidate as a free radical scavenger and antioxidant processor in an HCC rats model. This beneficial effect was achieved by antagonizing free radicals generation and the enhancement of the antioxidant defense mechanisms, resulting in marked improvement of hepatic biomarkers.

INTRODUCTION

Oxygen radical generation and lipid peroxidation have been implicated in the pathogenesis of various diseases and the toxic action of a wide range of compounds.^[1] Involvement of free radicals, generation of oxygen radicals and enhancement of lipid peroxidation have been shown to play an important role in hepatocellular carcinoma (HCC).^[2,3] Amelioration of the deleterious effects of oxidative stress associated with HCC using synthetic compounds causes undesirable side effects. Therefore, natural agents could be the most prudent strategy and the most effective agents for protecting humans from various diseases.^[4,5] Furthermore, there is the growing popularity of natural functional food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts.^[6,7]

One of the most important natural diets with antioxidant properties is berries, among the most widely consumed fruits in the human diet. Berry fruits, wild or cultivated, are proven as a traditional and rich source of bioactive compounds, possessing important biological substances such as flavonoids minerals, vitamins, and phenolic acids.^[8,9] One key berry fruit is, cape gooseberry (*Physalis peruviana*), a herbaceous plant which belongs to the Solanaceae family. Its fruit is also known as golden berry, ground cherry and in Egypt, harankash. The fruit of the cape gooseberry is highly nutritious, containing high levels of macronutrients and essential minerals such as magnesium, calcium, potassium, sodium, and phosphorus, as well as micronutrients such as iron and zinc,^[10,11] the fruit also contains vitamins A, B and C, in addition to β-carotene, α-carotene and β-cryptoxanthin. In addition, the fruit contains polyunsaturated fatty acids (e.g. linoleic acid and oleic acid). These bioactive compounds have nutritional value, medicinal properties, and an antioxidant property that can prevent peroxidative damage of liver cells.^[12-14] Cape gooseberry extracts show antioxidant activity,^[15,16] anti-inflammatory activity,^[17,18] and anti-hepatotoxic^[18] and anti-proliferative effects on hepatoma cells.^[19] This fruit

also has excellent potential as a food-based strategy for anti-diabetic and anti-hypertensive products.^[20]

Therefore, the objective of this study was to investigate the antioxidant properties of cape gooseberry juice as a potential source of natural functional substances against lipid peroxidation and scavenging capacities towards free radicals in different tissues of experimental HCC rats model.

METHODS

Chemicals

Diethylnitrosamine (DENA) and carbon tetra chloride (CCl₄) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DENA was freshly dissolved in sterile 0.9% saline and given to rats at a single dose of 200 mg/kg b.w.^[21] CCl₄ was given to rats at a dose of 3 mL/kg b.w. per week.^[22,23]

Animals

Healthy male albino rats (*Rattus rattus*), 8 weeks old (150-170 g) were purchased from Institute of Ophthalmic Disease Research, Cairo, Egypt. Rats were housed in cages at regulated temperature (22-25 °C). They were kept under good ventilation under a photoperiod of 12-h light/12-h darkness schedule with lights-on from 06:00 to 18:00. They all received a standard laboratory diet (60% ground corn meal, 10% bran, 15% ground beans, 10% corn oil, 3% casein, 1% mineral mixture and 1% vitamins mixture), purchased from Meladco Feed Company (Aubor City, Cairo, Egypt) and supplied with water *ad libitum* throughout the experimental period. Animals received humane care and the present study complies with the animal care guidelines. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

Hepatocellular rats model

Experimental hepatocellular carcinoma rats were subjected by a single intraperitoneal injection of freshly prepared DENA (200 mg/kg body weight), then 2 weeks later received a subcutaneous injection of CCl₄ once every week (3 mL/kg b.w.) for 10 weeks to

Table 1: Effect of CGJ administration on the tumor marker AFP and liver function enzymes activity in control and different treated rat groups (means ± SE)

Parameters groups	Serum				Liver		
	AFP (ng/mL)	ALT (U/mL)	AST (U/mL)	ALP (IU/L)	ALT (U/g)	AST (U/g)	ALP (U/g)
Control	0.99 ± 0.10	34.66 ± 1.02	40.16 ± 0.62	93.63 ± 0.38	60.77 ± 0.67	18.77 ± 0.34	33.03 ± 0.67
CGJ	1.00 ± 0.94	33.16 ± 1.06	41.00 ± 0.60	91.56 ± 0.50	59.99 ± 2.49	18.90 ± 0.33	32.14 ± 1.12
HCC	2.57 ± 0.28 ^{a,b}	50.50 ± 1.76 ^{a,b}	60.50 ± 0.99 ^{a,b}	153.11 ± 1.68 ^{a,b}	42.41 ± 0.51 ^{a,b}	15.16 ± 0.26 ^{a,b}	54.99 ± 1.12 ^{a,b}
HCC + CGJ	1.25 ± 0.66 ^{a,b,c}	37.33 ± 1.05 ^c	45.63 ± 0.63 ^{a,b,c}	105.65 ± 0.38 ^{a,b,c}	52.16 ± 0.61 ^{a,b,c}	16.98 ± 0.31 ^{a,b,c}	43.50 ± 1.10 ^{a,b,c}

^a $P \leq 0.05$ vs. control, ^b $P \leq 0.05$ vs. CGJ, ^c $P \leq 0.05$ vs. HCC. HCC: hepatocellular carcinoma; CGJ: cape gooseberry juice; AFP: alpha-fetoprotein; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase

promote the carcinogenic effect of DENA.^[23]

Preparation of cape gooseberry juice

Cape gooseberry (*Physalis peruviana*) was purchased from local markets at Mansoura, Egypt. Fruits were washed, cut into small pieces and freshly prepared juice [500 g cape gooseberry juice (CGJ) up to 500 mL distilled water, where each 1 mL juice contains 1 g cape gooseberry]. The cape gooseberry juice (1 mL/kg b.w.) was shaken well just before oral administration by gavage.^[17]

Experimental design

After 2 weeks of acclimatization, the rats were classified into 4 groups (6 rats/group) and treated for 12 weeks as follows: group 1 (control) rats did not receive any treatments; group 2 (CGJ): rats were orally administered with cape gooseberry juice (1 mL/kg b.w.); group 3 (HCC) rats were treated with a single intraperitoneal injection with DENA freshly dissolved in sterile 0.9% saline (200 mg/kg b.w.) and 2 weeks later given a subcutaneous injection of CCl₄ (3 mL/kg b.w. per week) for 10 weeks to promote the carcinogenic effect of DENA; group 4 (HCC + CGJ) rats were treated with DENA (200 mg/kg b.w.) and CCl₄ (3 mL/kg b.w. per week) plus CGJ (1 mL/kg b.w.).

Blood collection and tissue preparation

At the end of the experimental period (12 weeks), blood samples were collected from overnight rats, centrifuged at 860 g for 20 min at 4 °C and the separated sera were frozen at -20 °C for future biochemical analysis. Then the rats were sacrificed by cervical dislocation and the tissues (liver, kidney, brain and testes) removed and decapsulated. These tissues were weighed and homogenized in saline solution. The homogenates were centrifuged at 860 g for 20 min at 4 °C and the supernatants were frozen at -20 °C for further analysis.

Biochemical analysis

Alpha-fetoprotein (AFP) level was estimated by immunoenzymatic colorimetric method according

to Acosta.^[24] Aspartate transaminase (AST) activity, alanine transaminase (ALT) activity and alkaline phosphatase (ALP) were measured using colorimetric kits purchased by ABC Diagnostic Kit, Cairo, Egypt.^[25,26] Malondialdehyde (MDA) content was determined by the methods of Ohkawa *et al.*^[27] Reduced glutathione (GSH) was analyzed based on the method of Prins and Losse.^[28] Superoxide dismutase (SOD) and catalase (CAT) activities were assayed as described by Niskikimi *et al.*^[29] and Bock *et al.*^[30] respectively. Total antioxidant capacity (TAC) was determined using commercial Biodiagnostic kits (Dokki, Giza, Egypt) according to the methods of Koracevic *et al.*^[31]

Statistical analysis

Data were subjected to statistical significance tests using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test.^[32] The statistical analysis was carried out using SPSS 12.00 software. The results were expressed as mean ± SE and the differences were considered significant at $P \leq 0.05$.

RESULTS

The results of the present study [Table 1] recorded that the HCC rats treated with DENA and CCl₄ resulted in a significant increase in serum AFP level compared to the control level, indicating the development of HCC in rats. This elevation in AFP was accompanied by the elevation of serum and liver ALT, AST and ALP activity. The results in rats treated with CGJ alone were comparable to results in the control rats group in most of the estimated parameters. However, the administration of CGJ to the HCC rats was associated with a significant improvement in all the tested parameters where the treatment succeeded in reducing the elevation level of AFP, ALT, AST and ALP in both serum and liver [Table 1].

Moreover, the administration of CGJ to HCC rats succeeded in restoring oxidative stress through decreases in MDA level and induced a significant improvement in the antioxidant biomarkers by the observed increase in GSH, TAC, SOD and CAT in all

Table 2: Effect of CGJ administration on oxidative stress and antioxidant biomarkers in liver of control and different treated rat groups (means ± SE)

Parameters groups	MDA (nmol/g)	GSH (mg/g)	TAC (mmol/L)	SOD (U/g)	CAT (µmol/s/g)
Control	512.11 ± 0.64	19.46 ± 0.54	95.86 ± 0.03	892.99 ± 1.18	190.73 ± 1.19
CGJ	510.11 ± 0.59	19.99 ± 0.64	97.89 ± 0.13	894.90 ± 1.21	194.73 ± 1.89
HCC	701.80 ± 2.91 ^{a,b}	10.06 ± 0.46 ^{a,b}	28.11 ± 0.23 ^{a,b}	416.99 ± 1.18 ^{a,b}	132.55 ± 1.38 ^{a,b}
HCC + CGJ	517.11 ± 0.59 ^c	17.48 ± 0.44 ^{a,b,c}	75.47 ± 0.31 ^{a,b,c}	841.71 ± 1.77 ^{a,b,c}	180.68 ± 1.35 ^{a,b,c}

^a*P* ≤ 0.05 vs. control, ^b*P* ≤ 0.05 vs. CGJ, ^c*P* ≤ 0.05 vs. HCC. HCC: hepatocellular carcinoma; CGJ: cape gooseberry juice; MDA: malondialdehyde; GSH: glutathione; TAC: total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase

Table 3: Effect of CGJ administration on oxidative stress and antioxidant biomarkers in the kidney of control and different treated rat groups (means ± SE)

Parameters groups	MDA (nmol/g)	GSH (mg/g)	TAC (mmol/L)	SOD (U/g)	CAT (µmol/s/g)
Control	90.73 ± 1.12	42.56 ± 0.54	72.86 ± 0.23	69.99 ± 0.34	95.56 ± 1.54
CGJ	93.43 ± 1.16	42.99 ± 0.64	77.89 ± 0.13	70.79 ± 0.64	96.99 ± 0.64
HCC	190.93 ± 1.79 ^{a,b}	20.06 ± 0.46 ^{a,b}	20.21 ± 0.53 ^{a,b}	13.49 ± 0.66 ^{a,b}	30.16 ± 1.46 ^{a,b}
HCC + CGJ	105.77 ± 1.19 ^{a,b,c}	29.48 ± 0.44 ^{a,b,c}	37.47 ± 0.31 ^{a,b,c}	37.48 ± 0.94 ^{a,b,c}	70.48 ± 1.44 ^{a,b,c}

^a*P* ≤ 0.05 vs. control, ^b*P* ≤ 0.05 vs. CGJ, ^c*P* ≤ 0.05 vs. HCC. HCC: hepatocellular carcinoma; CGJ: cape gooseberry juice; MDA: malondialdehyde; GSH: glutathione; TAC: total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase

Table 4: Effect of CGJ administration on oxidative stress and antioxidant biomarkers in the brain of control and different treated rat groups (means ± SE)

Parameters groups	MDA (nmol/g)	GSH (mg/g)	TAC (mmol/L)	SOD (U/g)	CAT (µmol/s/g)
Control	75.31 ± 0.64	35.46 ± 0.50	75.06 ± 0.13	63.80 ± 0.34	62.43 ± 0.29
CGJ	76.44 ± 0.99	35.99 ± 0.64	77.00 ± 0.12	63.97 ± 0.64	62.43 ± 0.29
HCC	117.80 ± 1.71 ^{a,b}	13.76 ± 0.16 ^{a,b}	18.25 ± 0.12 ^{a,b}	18.25 ± 0.12 ^{a,b}	25.58 ± 1.70 ^{a,b}
HCC + CGJ	95.71 ± 0.99 ^{a,b,c}	23.48 ± 0.24 ^{a,b,c}	44.38 ± 0.94 ^{a,b,c}	44.38 ± 0.94 ^{a,b,c}	48.50 ± 1.05 ^{a,b,c}

^a*P* ≤ 0.05 vs. control, ^b*P* ≤ 0.05 vs. CGJ, ^c*P* ≤ 0.05 vs. HCC. HCC: hepatocellular carcinoma; CGJ: cape gooseberry juice; MDA: malondialdehyde; GSH: glutathione; TAC: total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase

Table 5: Effect of CGJ administration on oxidative stress and antioxidant biomarkers in the testes of control and different treated rat groups (means ± SE)

Parameters groups	MDA (nmol/g)	GSH (mg/g)	TAC (mmol/L)	SOD (U/g)	CAT (µmol/s/g)
Control	14.24 ± 0.02	25.02 ± 0.24	76.88 ± 1.23	50.04 ± 0.24	48.94 ± 0.12
CGJ	14.43 ± 0.06	25.28 ± 0.24	77.98 ± 1.13	50.22 ± 0.14	48.97 ± 0.24
HCC	54.96 ± 0.17 ^{a,b}	9.06 ± 0.16 ^{a,b}	35.55 ± 0.33 ^{a,b}	10.41 ± 0.22 ^{a,b}	16.03 ± 1.12 ^{a,b}
HCC + CGJ	31.95 ± 0.19 ^{a,b,c}	19.48 ± 0.04 ^{a,b,c}	57.77 ± 0.33 ^{a,b,c}	31.52 ± 0.23 ^{a,b,c}	30.48 ± 0.44 ^{a,b,c}

^a*P* ≤ 0.05 vs. control, ^b*P* ≤ 0.05 vs. CGJ, ^c*P* ≤ 0.05 vs. HCC. HCC: hepatocellular carcinoma; CGJ: cape gooseberry juice; MDA: malondialdehyde; GSH: glutathione; TAC: total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase

the examined tissues; liver, kidney, brain and testis, indicating the antioxidant activity of CGJ [Tables 2-5].

DISCUSSION

Recently, there has been growing interest in dietary bioactive compounds obtained from natural sources which have a therapeutic effect against various diseases including chemoprotective properties against cancer.^[5,33] HCC is a common disease, being the third leading cause of death worldwide.^[34] The current study suggests that treatment with DENA and CCl₄ is a good model for the induction of HCC in rats.^[35] The data also showed increased AFP in HCC rats. Increase of this protein may be due to hepatotoxic agents or hepatocarcinogens that are frequently associated with HCC. Increased glycoprotein AFP levels is considered

a good marker for various malignancies including testicular, bile duct, pancreatic, stomach, colon and hepatic cancer.^[36,37] Moreover, the observed elevation of serum AST, ALT and ALP and the decrease in ALT and AST in the liver in HCC rats supports earlier findings.^[38] These findings may be due to damage to hepatocytes caused by exposure to DENA resulting in hepatic dysfunction and subsequent leakage of these enzymes from the neoplastic cell into circulation.^[39] Or, the findings may be due to the release of enzymes from normal tissue by tumors or possibly the effect of tumors on remote tissue, leading to leakage of enzyme and release into the blood.^[40] In a related concern it has been suggested that there is an increase in the levels of these transaminases activity in serum of HCC patients.

In concurrence with the above findings, elevated serum

aminotransferase activity is more specific for liver injury due to damage to the liver cell membrane.^[40] As well, alkaline phosphatase is used as a specific tumor marker for making diagnoses in the early detection of cancer.^[41] This enzyme is involved in the transport of metabolites across cell membranes, in protein synthesis, secretory activities and glycogen metabolism. It is a membrane-bound enzyme, and its alteration is likely to affect the membrane permeability that produces derangement in the transport of metabolites.^[42]

The observed increases of serum and liver ALP in HCC rat groups may be due to altered gene expression.^[43] In the current study, the HCC rats group suffered from severe oxidative stress in various organs, achieved by elevation of MDA level and depletion of antioxidant enzymes. This may be due to the conversion of cellular poly-unsaturated fatty acids to the toxic product MDA which has a cytotoxicity and inhibitory action on cellular protective enzymes.^[44] HCC caused by carcinogenic DENA generally reflects instability of liver metabolism associated with free radicals species (ROS) generation, which leads to oxidative stress and alterations in antioxidant defense mechanisms.^[35,45] Increased level of MDA has been reported during DENA-induced hepatocarcinogenesis. This dynamic action may further lead to uncompromised production of free radicals overwhelming the cellular antioxidant defense.^[46,47] Moreover, HCC causes depletion of SOD and CAT activity as well as TAC and GSH contents in all observed organs. Such studies support the current findings, as the current study showed a significant decrease in the activities of antioxidant enzyme in the liver of animals treated with carcinogen.^[35] Antioxidants are substances that either directly or indirectly protect cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions.^[48] The observed decrease in SOD activity in liver, kidney, brain and testes suggests the inactivation of antioxidant enzymes; this is possibly due to increased superoxide radical production or to an inhibition by H₂O₂ as a result of corresponding decrease in the activity of catalase which selectively degrades H₂O₂.^[49] The decreased GSH, SOD and CAT observed in the HCC group of rats may be due to accumulation of lipid peroxidation that was seen to increase during carcinogenesis.

The accompanying reduction in the activity of SOD, CAT and depletion of GSH content suggests induction of oxidative stress in the organs studied. SOD is considered the first line of defense against deleterious effects of oxygen free radicals in the cells by catalyzing of superoxide radicals (O²⁻) to H₂O₂ and molecular oxygen. CAT is also responsible for the detoxification

of H₂O₂, which is an effective inhibitor of SOD.^[50,51] The reduction in the activity of CAT may reflect the inability of tissues to eliminate H₂O₂. CAT protects SOD against inactivation by H₂O₂, while SOD protects CAT against inhibition by (O²⁻). Thus, the balance of this enzyme system may be essential to eliminating ROS generated in the tissues. In this area, GSH represents an important defense mechanism in protecting cells against ROS.^[52] On the other hand, the enormous impacts of CGJ supplementation in alleviating oxidative stress in all organs in the HCC rats may be attributed to either a direct effect of many antioxidant compounds of CGJ as free radical scavengers, or to enhancement of cellular antioxidant defense functions. This occurs through modulating the alteration in GSH content and antioxidant enzymes activity. An amelioration of AFP after supplementation of CGJ to HCC rats may be due to the antioxidant activity of CGJ. Additionally, the observed increase in alterations to liver enzymes, including ALT, AST and ALP, in HCC-received CHJ rats may be due to the improvement of the functional status of hepatocytes with preservation of cellular architecture leakage of intracellular enzymes through membrane-stabilizing activity.^[18,53]

Previous studies have suggested that CGJ is a significant source of natural antioxidative compounds.^[54] These components may have a wide variety of chemical structures that could react with radicals by donating protons (free radical quenching), radical addition, redox reaction (electron transfer) and radical combination.^[12] Natural antioxidants could prevent the deleterious effects of toxic agents by scavenging free radicals and other ROS or by modulation of the inflammatory response. The supplementation of CGJ to the HCC rats model resulted in amelioration of oxidative stress. The improvement of the antioxidants defense mechanism is considered a favorable indicator for anti-lipid peroxidative properties and antioxidant activity through high levels of antioxidant compounds such as polyphenols and similar flavonoids.^[55,56]

The observed decreases in MDA in HCC rats that received CGJ may be due to free radicals scavenging, a potential mechanism by which CHJ can act as an anti-inflammatory and antioxidant to protect the liver and other organs. Therefore, dietary consumption of cape gooseberry may be a highly effective potential antioxidant and protective agent against oxidative stress in liver toxicity.^[57,58]

In view of the present results, it was observed that CGJ supplementation showed a significant antioxidant status as manifested by elevation of GSH, TAC, SOD and CAT in serum and various organs. Many plant

secondary metabolites act as potent antioxidants and it has been demonstrated that free radical scavenger/antioxidants such as SOD, CAT, TAC and reduced glutathione (GSH) prevent the tissue damage induced by different toxicants.^[59] The first line of defense against superoxide anion (O_2^-), H_2O_2 and (OH), the major ROS which induce cell degeneration by increasing LPO of cell membrane lipids, is the family of enzymes SOD and CAT that convert O_2^- to H_2O_2 . The toxic end products of peroxidation induce damage to the structural and functional integrity of cell membranes, break DNA strands, and denature cellular proteins. The natural cellular antioxidant enzyme SOD is an important enzyme as because it is found virtually in all aerobic organisms. O_2^- is the only known substrate for SOD which is considered to be a stress protein, which is synthesized in response to oxidative stress.^[60]

In conclusion, there is a significant relationship between HCC and free radical-mediated oxidative stress demonstrated by increased levels of MDA as well as decreased levels of anti-oxidant parameters in the examined organs of rats. The obtained data also strongly suggested the antioxidant activity of cape gooseberry supplementation, as evidenced by the greatly positive effect on reduced oxidative stress as well as improvement in the cellular anti-oxidant defense system antioxidant status. The underlying mechanisms for this protective effect may be through various nutritional constituents due, at least in part, to their synergistic anti-oxidant capacity as well as scavenging free radicals. Thus, blocking the oxidative stress pathway may be of therapeutic value in treatment of liver injury. These results suggest that CGJ-enriched diets should be added to diet regimens to develop a new therapeutic strategy for treatment of diseases associated with free radicals generation. The fractionation and bioavailability of the main constituents of cape gooseberry, which are responsible for the anti-oxidant activity, will be an important area of study in the future.

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Conflicts of interest

There are no conflicts of interest.

Patient consent

There is no patient involved.

Ethics approval

The local committee approved this study and the protocol conforms to the guidelines of the National Institutes of Health.

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