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Original article

Effects of polyphenols of *Cocos nucifera* husk fibre on selected indices of cardiovascular diseases in mice

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ABSTRACT

Cocos nucifera (*C. nucifera*) oil is indigenously used to treat cardiovascular diseases. However, coconut husk fibre (which is rich in polyphenols) has not been screened for this property. Based on the ethnomedicinal use of polyphenols in treating cardiovascular diseases. this study was carried out to evaluate the effects of polyphenols of *C. nucifera* husk fibre on selected cardiovascular disease indices in mice. Fifty adult male Swiss albino mice were assigned randomly into five groups (A-E). Mice in groups B, C, D and E were administered 31.25, 62.5, 125, and 250 mg/kg body weight polyphenols of ethyl acetate extract of *C. nucifera* husk fibre respectively while the control group (A) mice received 5% DMSO for seven days. The mice were sacrificed twenty four hours after the last administration of polyphenols. Heart and plasma lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities and plasma lipid profile were determined. Results revealed significant reduction ($^{*}p < 0.05$) in plasma levels of total cholesterol and LDL-cholesterol with no significant change ($^{*}p > 0.05$) in HDL-cholesterol, triglyceride and VLDL levels in the plasma at all doses of polyphenols administered compared to controls. There was significant reduction ($^{*}p < 0.05$) in the activities of heart AST and LDH while plasma ALT, AST, and ALP activities were not significantly altered ($^{*}p > 0.05$) at all doses of polyphenols administered compared to controls. These results suggest that the polyphenols of *C. nucifera* husk fibre possess cardio-protective properties and also indicate their possible use in the treatment of cardiovascular diseases.

Keywords coconut, polyphenols, cardiovascular diseases, husk fibre

INTRODUCTION

Cardiovascular diseases are the leading cause of death globally (Anthea et al., 1993). About 80% of deaths arising from cardiovascular diseases occur in under-developed and developing countries. WHO (2012) estimated that 23.6 million people will die from cardiovascular diseases by 2030, if current trends are allowed to continue. Most people living in developing countries are almost completely dependent on herbal remedies for the treatment of these diseases due to their cheaper cost and the historical/cultural beliefs of the people (Agner et al., 2001; Prozesky et al., 2001).

The search for novel drugs from plants used in folkmedicine for the treatment of cardiovascular diseases could lead to new strategies for reducing the risk of the diseases. DebMandal and Mandal (2011) reported that *Cocos nucifera* Linn. (coconut, *C. nucifera*) water had protective effect against myocardial infarction, which is mainly due to its high potassium content. Coconut oil is indigenously used for the treatment of cardiovascular diseases. This has been scientifically authenticated by Nevin and Rajamohan (2004), who reported that virgin coconut oil reduced total cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-C) but increased high density lipoprotein cholesterol (HDL-C). Polyphenols have been reported to be effective in the treatment of cardiovascular diseases (Han et al., 2007). Polyphenolic fraction of virgin coconut oil has been demonstrated to prevent oxidation of LDL *in vitro* (Nevin and Rajamohan, 2004). Polyphenols of the husk fibre of *C. nucifera*, a medicinal plant used in the North Central region of Nigeria, have been reported to be responsible for the antioxidant, antimicrobial, antineoplastic, antileishmanial and antimalarial activities of the husk fibre (Adebayo et al., 2013; Alviano et al., 2004; Mendonca-Filho et al., 2004). Some of the identified polyphenols of *C. nucifera* husk fibre are catechin, epicatechin and epicatechin-(4→2)- phloroglucinol (Esquenazi et al., 2002). These phytochemicals are potent antioxidants which scavenge prooxidants and free radicals (Joyex et al., 1995). Catechins have been reported as powerful inhibitors of cellular growth (Chen et al., 1988; Paschka et al., 1998; Yang and Wang, 2010), possessing anticancer (Barthelman et al., 1998; Fujiki et al., 1998), antimutagenic (Geetha et al, 2004), antibacterial (Newton et al., 2002) and anti-inflammatory activities (Bighetti et al., 1999; Yang et al., 1998).

However, there is paucity of information on the effects of polyphenols of *C. nucifera* husk fibre on indices of cardiovascular diseases. This study was therefore aimed at evaluating the effects of polyphenols of *C. nucifera* husk fibre on selected cardiovascular disease indices in mice.

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MATERIALS AND METHODS

Chemicals

Absolute n-Hexane, Ethyl acetate, α-Ketoglutarate, ATP, NAD^+, Sodium Pyruvate, 2, 4-Dinitrophenylhydrazine, para-NitroPhenol Phosphate (PNPP) and Sodium L-Lactate were obtained from Sigma-Aldrich Laborchemikalien GmbH, Germany. Disodium Hydrogen Phosphate and Potassium Dihydrogen Phosphate were obtained from Kermel Chemicals, China. Sodium bicarbonate, L-glutamine, L-glycine, glucose, Tris HCl, sodium hydroxide and magnesium sulphate were obtained from BDH laboratory Supplies, Poole, BH 15 1TD, England. Assay kits for plasma analysis were obtained from Randox Laboratories Ltd, UK. Other reagents were of analytical grade and were prepared in all glass distilled water.

Animals

Fifty adult male Swiss albino mice (*Mus musculus*; average weight of 20 ± 0.57 g) were obtained from the Animal Holding Unit of the Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Nigeria. The animals were housed in standard plastic cages and were maintained under standard conditions (12 h light/dark cycle, room temperature: 28°C - 31°C, Humidity: 50-55%). The mice had access to feed (Top Feeds Ltd, Nigeria) and water *ad libitum*.

Plant materials

Husk fibres of *Cocos nucifera* (West African Tall variety - WAT) dried at room temperature under shade were obtained from Nigeria Institute for Oil Palm Research (NIFOR), Badagry, Lagos State, in January, 2013. It was taxonomically authenticated at the Herbarium in the Department of Plant Biology, where a voucher specimen was deposited with a Voucher Number UIH001/508.

Ethical approval

Ethical approval for the study was obtained from the postgraduate committee of the Department of Biochemistry, University of Ilorin, Nigeria.

Preparation of extract

The method of Adebayo et al. (2003) was used for preparing the extract. The samples were dried under shade at room temperature and pulverized into powder. The powder (2 kg) was percolated in 12.75 l of n-hexane for 72 h in a tightly stoppered glass container. This was shaken at intervals. The resulting mixture was filtered with Whatmann filter paper (110 mm). The filtrate was then concentrated at 40°C under pressure using rotary evaporator, thereby generating the crude hexane extract. The residue was air dried, to allow complete evaporation of the n-hexane from the sample. This residue was again percolated in 12.75 L of ethyl acetate for another 72 h. This was filtered using Whatmann filter paper No. 1 filter paper and the filtrate was likewise concentrated at 40°C using a rotary evaporator. This generated the ethyl acetate extract which was subsequently used for the experiment. The percentage yield was 0.26%.

Extraction of polyphenols

The method described by Boham and Kocipai (1974) was used to extract polyphenols from the ethyl acetate extract.The ethyl acetate extract (5 g) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath at 37°C to a constant weight and the yield was 0.0265%.

Confirmatory phytochemical screening

The polyphenolic fraction was screened for the presence of tannins and flavonoids according to the methods described by Sofowora (1993) and Harbourne (1973).

Animal grouping and administration of extract

The fifty mice were assigned randomly into five groups (A - E), of 10 mice each per cage. Group A mice were orally administered 0.2 ml of 5% dimethyl sulfoxide solution while groups B, C, D, and E mice were orally administered 31.25, 62.5, 125 and 250 mg/kg body weight polyphenolic fraction of ethyl acetate extract of *C. nucifera* husk fibre respectively for seven days. The polyphenolic fraction was dissolved in 5% DMSO solution to form a homogenous suspension before administration.

Collection and preparation of samples

Twenty-four hours after the last set of doses were administered, the mice were slightly anaesthetized using diethyl ether. Their neck area was cleared of fur and the jugular veins exposed, from which blood was collected into EDTA bottle to prevent clotting. The blood samples were centrifuged at 403 xg for 10 min and the plasma pipetted out. This was stored frozen until needed for analysis. The mice were also dissected to isolate the heart, which was cleaned of blood, suspended in ice-cold 0.25 M sucrose solution (1:5 w/v) and homogenized. This was stored frozen at -20°C overnight for complete cell lysis and maximum release of enzymes.

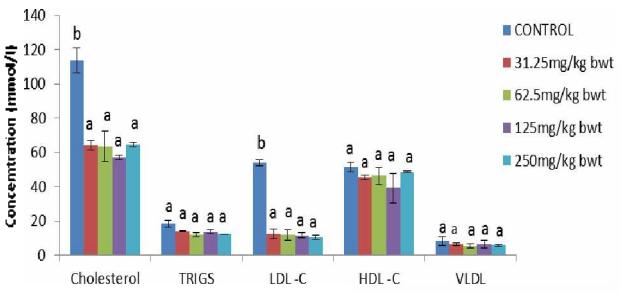

Fig. 1. Plasma lipid profile of mice administered polyphenols of *Cocos nucifera* husk fibre. Values are means ± SEM of 10 replicates. Values for each parameter with different superscripts (a, b) are significantly different ($^*p < 0.05$) from each other. TRIGS = Triacylglycerol, LDL-C = Low density lipoprotein-cholesterol; HDL-C = High density lipoprotein-cholesterol; VLDL = Very low density lipoprotein.

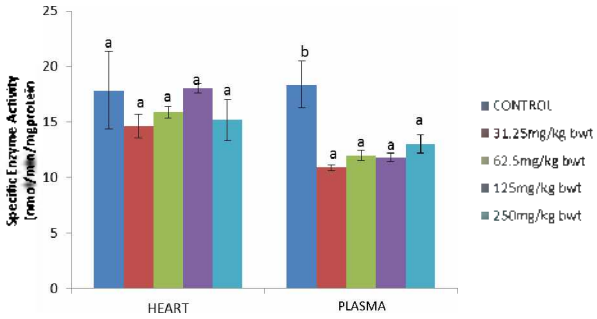

Fig. 2. Alkaline Phosphatase activities in heart and plasma of mice administered polyphenols of *Cocos nucifera* husk fibre. Values are means ± SEM of 10 replicates. Values for the heart/plasma with different superscripts (a, b) are significantly different ($^*p < 0.05$) from each other

Table 1. Effect of Polyphenols of *Cocos nucifera* husk fibre on Heart to Body Weight Ratio (%) of mice

Treatment (mg/kg body weight)	Heart/ Body Weight (%)
Control (5% DMSO)	0.47 = 0.05[a]
31.25	0.55 = 0.06[a]
62.5	0.58 = 0.05[a]
125	0.44 = 0.04[a]
250	0.49 = 0.03[a]

Values are means ± SEM of 10 replicates. Values with the same superscripts (a. b) are not significantly different ($^*p > 0.05$) from each other.

Table 2. Atherogenic index of mice administered polyphenols of *Cocos nucifera* husk fibre

Groups	Atherogenic index	
	Total Cholesterol / HDL- Cholesterol	LDL-Cholesterol / HDL- Cholesterol
Control	2.23 ±0.20[b]	1.06±0.20[b]
31.25 mg/kg B wt.	1.42 =0.03[a]	0.28±0.03[a]
62.5 mg/kg B wt.	1.38 =0.07[a]	0.26±0.07[a]
125 mg/kg B wt.	1.57 =0.26[a]	0.39±0.21[a]
250 mg/kg B wt.	1.33 =0.04[a]	0.22±0.03[a]

Values are means ± SEM of 10 replicates. Values in the same column with different superscripts (a. b) are significantly different ($^*p < 0.05$) from each other.

Determination of heart-body weight ratio

The percentage heart-body weight ratio was calculated thus: Percentage Heart to Body Weight.

$$= \frac{Fresh\ Weight\ of\ Heart\ (g)}{Weight\ of\ Animal\ (g)} \text{ X } 100$$

Biochemical Assays

The protein contents of the plasma and homogenate were determined using the method described by Gornall et al. (1949). Alkaline phosphatase activity was determined by the method of Wright et al. (1972). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the method of Reitman and Frankel (1957). Lactate dehydrogenase activity was determined by the method described by Wroblewski and La due (1955), with slight modification. The modification includes measuring the amount pyruvate produced instead of NADH by adding 0.5 ml of 0.001 M DNPH at the end of the reaction and allowing it to stand for 15 min, after which reaction was terminated by the addition of 5.0 ml of 0.4 N NaOH and absorbance read at 440 nm. The method described by Allain et al. (1974) was used in determining plasma total Cholesterol concentration while concentrations of triglycerides and HDL-C in the plasma were determined using the methods described by Tietz (1990) and Bachorik et al. (1980) respectively. LDL-C and VLDL concentrations were calculated using the formula described by Friedwaldet al. (1972). Atherogenic index was calculated using the formula described by Lamarche et al. (1996).

Statistical analysis

Experimental data are presented as Means ± Standard error of mean (SEM). Statistical analysis was implemented using SPSS 20.0 version statistical package program (SPSS, Chicago, IL). One-way analysis of variance was used to compare variables among the different groups. Level of significance (Post hoc comparisons) among the various treatments was determined by Duncan's Multiple Range Test. The differences were considered statistically significant at $^*p < 0.05$.

RESULTS

Administration of polyphenols of *C. nucifera* husk fibre at all doses investigated in this study did not cause any significant change in the heart-body weight ratio compared to control (Table 1).The polyphenols of *C. nucifera* husk fibre at all doses administered in this study did not cause any significant alteration in plasma triglyceride, HDL-C and VLDL concentrations compared to controls while they significantly reduced plasma LDL-C and total cholesterol concentrations at all doses administered compared to controls (Fig. 1). The polyphenols of *C. nucifera* husk fibre reduced the atherogenic index significantly ($^*p < 0.05$) at all doses administered compared to control Polyphenols of *C. nucifera* husk fibre did not cause any significant change in heart ALP activity while they reduced plasma ALP activity significantly ($^*p < 0.05$) at all doses administered compared to controls (Fig. 2). There was significant reduction ($^*p < 0.05$) in heart AST activity with no significant alteration in plasma AST activity at all doses of polyphenols of *C. nucifera* husk fibre compared to controls (Fig. 3). There was no significant change in plasma ALT activities of mice administered different doses of polyphenols of *C. nucifera* husk fibre compared to control (Fig. 4). Heart lactate dehydrogenase (LDH) activity was reduced significantly at all doses of polyphenols of *C. nucifera* husk fibre administered compared to control (Fig. 5, $^*p < 0.05$).

DISCUSSION

Various parts of *C. nucifera* fruit have been demonstrated to possess diverse pharmacological activities. Coconut water has been reported to possess cardioprotective (Rajamohan et al., 2003), renal protective (Gandhi et al., 2013), hepatoprotective and antioxidant (Loki et al., 2003) activities. *C. nucifera* endocarp has been demonstrated to possess vasorelaxant and antihypertensive activities (Bankar et al., 2011). *C. nucifera* husk fibre has been reported to exhibit antimicrobial (Jose et al., 2014), antimalarial (Adebayo et al., 2013), antitrichomonal (Cedillo-Rivera et al., 2002), and anti-leishmanial (Mendonca-Filho et al., 2004) activities. However, this is the first time the husk fibre of the fruit is being evaluated for its effect on some indices of cardiovascular diseases.

Changes in organ-body weight ratio may be an indication of organ constriction or inflammation. The constriction in the organ may occur as a result of loss of fluid from the organ due to damage, while increase in organ-body weight ratio may suggest inflammation (Moore and Dalley, 1999). Therefore, since there was no change caused by polyphenols of *C. nucifera* husk fibre in the heart-body weight ratio of mice in this study, it thus suggests that the polyphenols may not cause constriction or inflammation in the heart.

Alterations in the concentrations of the main lipids in the blood can give useful information on lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Yakubu et al., 2008). The results revealed that the polyphenols at all doses administered reduced total cholesterol concentration in the plasma. This

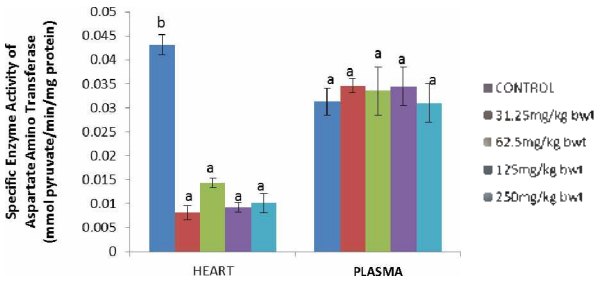

Fig. 3. Aspartate aminotransferase activities in heart and serum of mice administered polyphenols of *Cocos nucifera* husk fibre. Values are means ± SEM of 10 replicates. Values for heart/plasma with different superscripts (a, b) are significantly different (*$p < 0.05$) from each other.

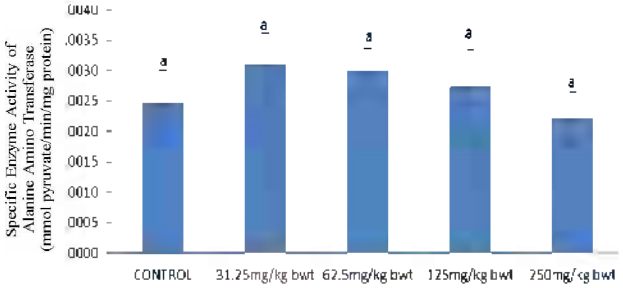

Fig. 4. Alanine aminotransferase activities in plasma of mice administered polyphenols of *Cocos nucifera* husk fibre. Values are means ± SEM of 10 replicates. Values with the same superscript (a, b) are not significantly different (*$p > 0.05$) from each other.

finding corroborates earlier report by Nevin and Rajamohan (2004) who stated that virgin coconut oil (also containing polyphenols) obtained from the same plant reduced total LDL cholesterol and cholesterol concentrations compared to controls. The observed reduction in total cholesterol concentration may be due to increased catabolism and excretion of cholesterol or reduced synthesis of cholesterol. Certain drugs/herbs have been reported to cause enhanced excretion of acidic and neutral steroids (Nurminen et al., 1998; Udoh, 1998). The decrease in cholesterol may also result from enhanced enterohepatic circulation through sequestration effected by the polyphenols (Onyeike et al., 2012).

Triglycerides and very low density lipoprotein have clinical importance in assessing coronary heart diseases, although Rapaport et al. (1993) could not establish increase in plasma triglyceride level as an independent risk factor. The polyphenols administered did not have any adverse effect on the concentrations of triglycerides and very low density lipoprotein in the plasma which suggests that they may not adversely affect triglyceride metabolism. Low density lipoprotein transports cholesterol to peripheral tissues where they are taken up and used by cells (Belal, 2011). High level of LDL-cholesterol in the plasma is linked with increased deposition of cholesterol in the arterial walls (Vander et al., 1998). Reduction in LDL-cholesterol concentration by 2 mg/dl or 0.1 mmol/l can result in 1% reduction in the risk for coronary artery disease (Khanna et al., 2002). The observed decrease in concentration of LDL- cholesterol at all doses of polyphenols administered compared to control suggests increased uptake of LDL-cholesterol at the peripheral tissues, possibly by increasing the LDL-C receptor densities in the tissues and enhancing the binding to apolipoprotein (Baum et al., 1998). This may prevent the deposition of cholesterol in arterial walls and the subsequent LDL oxidation because polyphenolic fraction of virgin coconut oil has been reported to prevent LDL oxidation *in vitro* (Nevin and Rajamohan, 2004).

This suggests cardio-protective effect of the polyphenols. HDL mediates the removal of cholesterol from peripheral tissues to the liver, thereby facilitating its catabolism in the liver and subsequent secretion of the catabolic products into the bile (Vander et al., 1998). The polyphenols did not alter plasma HDL-cholesterol concentration, suggesting that they may not adversely affect HDL metabolism and thus they may not pose any risk of coronary heart disease. The best single indicator of the likelihood of developing atherosclerotic heart disease is not total plasma cholesterol but rather the ratio of the plasma total cholesterol to HDL-C (atherogenic index) which has been shown to be 68% sensitive and 98% specific or a ratio of the LDL-C to HDL-C which is 76% sensitive and 99% specific (Khazaal, 2013; Demosthenes et al., 2003). Myocardial infarction increases considerably when the ratio is higher than 5 (Boers et al., 2003; Steiner and Li, 2001). The reduced atherogenic index observed in the groups treated with polyphenols compared to control suggests that polyphenols of *C. nucifera* husk fibre possess anti-atherosclerotic potential and may not predispose subjects to cardiovascular diseases.

Alkaline phosphatase (ALP) has been reported to be a marker enzyme for assessing the integrity of plasma membrane and endoplasmic reticulum (Akanji et al., 1993; Wright et al., 1972). Reduction of ALP activity in the tissue with a corresponding increase in serum ALP activity would indicate damage to the plasma membrane (Yakubu, 2006). The results revealed that the polyphenols at all doses administered did not cause any significant change in heart ALP activity. Therefore, the polyphenols of *C. nucifera* husk fibre may not have any adverse effect on the integrity of the plasma membrane of cardiomyocytes. Thus, observed reduction in serum ALP activity at all doses of polyphenols administered compared to control might be due to inactivation of the enzyme *in situ* by the polyphenols (Adebayo et al., 2013).

ALT and AST are involved in the transfer of amino groups from α-amino acids to α-keto acid, thereby performing a significant role in the regulation of intracellular amino acid pool. The activities of the enzymes are increased in the plasma in conditions in which organs such as the liver and heart are damaged (Jimoh and Odutuga, 2001), though AST activity in the plasma is a more specific indicator of heart damage affecting cell membrane integrity (Prabodhet al., 2012). The polyphenols of *C. nucifera* husk fibre did not alter ALT and AST activities in the plasma, suggesting there was no leakage from the tissues into extracellular fluids. However, heart AST activity was reduced at all doses of the polyphenols administered in this study, suggesting inhibition of the enzyme *in situ* or reduced synthesis of the enzyme (Adebayo et al., 2013).

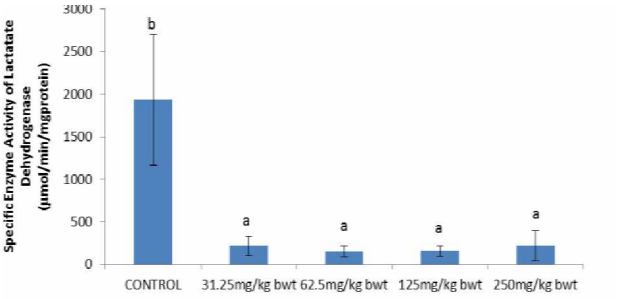

Fig. 5. Lactate Dehydrogenase activities in heart of mice administered polyphenols of *Cocos nucifera* husk fibre. Values are means ± SEM of 10 replicates. Values with different superscripts (a, b) are significantly different (*$p < 0.05$) from each other.

LDH is a cytoplasmic enzyme that is essential in the conversion of lactate to pyruvate (Murray et al., 2007). Under conditions of stress, anaerobic glycolytic pathway is activated and high levels of lactate are produced from pyruvate in the cell and thus an increase of LDH activity is expected (Kumaret al., 2010). Increased lactate dehydrogenase activity in the heart and blood has been associated with myocardial infarction. The reduction observed in heart lactate dehydrogenase activity at all doses of polyphenols administered suggests an enhancement of aerobic glycolysis in the heart cells (Adebayo et al., 2013), thus reducing the risk of myocardial infarction.

In conclusion, the results of this study suggest that polyphenols of *C. nucifera* husk fibre possess anti-hypercholesterolemic potential. Moreover, the polyphenols may enhance complete oxidation of glucose in cardiac cells and may not adversely affect the plasma membrane of the cardiac cells. Thus, polyphenols of *C. nucifera* husk fibre may reduce the risk of myocardial infarction and atherosclerosis.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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