Comparative Safety Evaluation of *Alstonia boonei* and *Sphenocentrum jollyanum* on High Fat Diet Induced Obesity in Male Wistar Rats

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Abstract

This study was designed to compare the effect of ethanolic extract of *Alstonia boonei* stem bark and *Sphenocentrum jollyanum* root on high fat diet induced obesity in male wistar rats. Thirty two male wistar rats weighing 85 ± 5 g were randomly assigned into four groups. Group 1 was fed the normal pellet diet (NPD) and groups 2 – 4 were fed high fat diet (HFD) and water *ad libitum* for 18 weeks. Treatment commenced on the 14th week, group 2 received no treatment while groups 3 and 4 received 500mg/kg b.w orally of *A. boonei* and *S. jollyanum* extracts respectively for 4 weeks. Weekly body weights and BMI of all the rats were measured. At the end of 18 weeks, creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD) and catalase (CAT) activities were determined. Also, creatinine, urea, uric acid, malondialdehyde (MDA) and reduced glutathione (GSH) levels were measured. *A. boonei* significantly decreased (P<0.05) body weight, BMI, urea, uric acid, ALT, AST and ALP levels, CK and LDH activities, while *S. jollyanum* significantly decreased (P<0.05) the BMI, ALT, AST and ALP levels and CK activities when compared with the HFD obese control. Both *A. boonei* and *S. jollyanum* treated groups showed significant increase (P<0.05) in catalase, SOD and GSH activity and no significant decrease in MDA level. Thus, the ethanolic extracts of *A. boonei* showed more promising antiobesity potential than *S. jollyanum* in rats fed high fat diet.

Key words: *Alstonia boonei*, *Sphenocentrum jollyanum*, antiobesity, high fat diet, body mass index

Introduction

When we eat more calories than we burn, our bodies store this extra energy as fat, which leads to weight gain, overweight, and ultimately obesity. Obesity is often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue (WHO, 2000). Obesity may lead to serious health problems, including high blood pressure, heart disease, stroke, type 2 diabetes, kidney disease, fatty liver disease (also called nonalcoholic steatohepatitis or NASH) and certain cancers. In fact, obesity is one of the leading preventable causes of death worldwide (Barness *et al.,* 2007; Mokdad *et al.,* 2004; Allison *et al.,* 1999).

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Obesity may be defined as a body mass index (BMI) of 30 and above (WHO, 2000). BMI is one way to tell whether you are at a normal weight, overweight, or obese. The BMI measures your weight in relation to your height. Almost 70% of adults in the U.S.A. are overweight but, perhaps more alarmingly, 16% of juveniles are overweight (Albrecht *et al.,* 2004). Among Americans age 20 and older, 154.7 million are overweight or obese (BMI of 25.0 kg/m2 and higher): 79.9 million men and 74.8 million women. Of these, 78.4 million are obese (BMI of 30.0 kg/m2 and higher): 36.8 million men and 41.6 million women (Go *et al.,* 2013).

In Africa and other developing countries, there has been an acute transition from traditional to a westernized or modern-world life-style characterized by consumption of high fat diets, sedentary living, etc. For instance, in Nigeria (West Africa) fast food outlets have increased in the last 15 years. In Nigeria, although there is paucity of national statistics on obesity, community studies have been conducted by researchers. Studies have shown the prevalence of obesity, and higher prevalence rates of obesity among females compared with males have been observed (Iloh *et. al.,* 2011; Bakari *et. al.,* 2007).

Treatment of obesity involves the use of drugs like orlistat, however, to stop the current trends in the growth of obesity, scientist must continue the research for cheaper, safer, faster and locally available means to treat obesity. Traditional medicinal plants are often cheaper, locally available, and easily consumable (raw or as simple medicinal preparations). Some traditional healers have claimed that some medicinal plants in Nigeria like *Alstonia boonei* and *Sphenocentrum jollyanum* could be used to treat obesity.

Alstonia boonei is a tree of 30-40 m high by over 3 m in girth, bole cylindrical and long to 27 m, with high narrow buttresses. *A. boonei* is a medicinal plant used extensively in West and Central Africa for the treatment of malaria, fever, intestinal helminthes, rheumatism, hypertension, etc. (Terashima, 2003; Betti, 2004; Abel and Busia, 2005). *Sphenocentrum jollyanum* is a deep rooted plant that grows up to 1.5m high having few branches. It has been shown to display a wide spectrum of biological and pharmacological activities. The medicinal importance of the plant was first reported by Dalziel (1955) in which it was noted that the leaves decoctions were used as vermifuge. It is reputed for use in dressing wounds. The plant is also used for treating feverish conditions, cough, jaundice, breast swelling related to menstrual cycles, as an aphrodisiac and other inflammatory conditions such as tumours (Iwu, 1993). Therefore, the aim of this study was to investigate the comparative evaluation of *A. boonei* and *S. jollyanum* on high fat diet induced obesity in wistar rats

MATERIALS AND METHODS
Plant Material

Fresh stem bark of *A. boonei* De Wild and *S. jollyanum* Pierre were collected from a farm land located in Umuekwune community, Imo State, Nigeria. The authentication of the plants was done by Ihuma, J. O., Department of Biological Sciences, Bingham University, Nigeria.

Preparation of Plant Extract

The fresh parts of the plants was washed, chopped into pieces and air-dried in the laboratory at room temperature. The dried plants parts was milled into powder and weighed. 250g of each of the plant powder was soaked in 500 ml of absolute ethanol (analytical grade purchased from Pyrex chemical company, Benin

Induction of Obesity in the Rats

city) separately in Schott Duran bottles for 72 hours with intermittent shaking. Then, each portion was filtered through Whatman No. 1 filter paper. The resulting filtrates were evaporated under reduced pressure using a rotary evaporator and there after freeze dried to obtain powder. The yield was stored in a refrigerator (4 °C) till when needed.

Phytochemical Screening

Phytochemical screening of the stem bark of *A. boonei* De Wild and *S. jollyanum* Pierre was done using standard methods described by Treatise and Evans (1989) and Sofowora (1993).

Acute Toxicity (LD$_{50}$) of Plants

The acute toxicity of the ethanolic extract of the plants was carried out as described by Shah *et. al.* (1997) and Burger *et. al.* (2005). Thirty five albino rats were divided into seven groups of five (5) rats each weighing between (180-200 g). The rats were subjected to 24 hour fasting (with only water) before administering the extract. The extract was suspended in distilled water and administered in doses of 200, 400, 800, 1600, 3200 and 6400 mg/kg body weight orally. The seventh group served as control and will receive only distilled water. The rats will be observed for signs of toxicity and mortality for the first critical 4 hours and then for each hour for the next 12 hour, followed by 6 hourly intervals for the next 56 hour giving a total of 72 hour observations, thereafter daily for 7 days.

Experimental Animals

Thirty two male albino rats weighing 85 ± 5 g were used for this study. The rats were purchased from the Anatomy Department, University of Benin, Nigeria. All animals were housed in steel cages and each cage contained 8 rats. Rats were maintained under controlled temperature (± 23°C) and a 12:12 h light/dark cycle. The rats were housed for two weeks after their arrival to the animal house for acclimatization. The rats had free access to tap water and normal pellet diet (NPD) until they were assigned to individual groups. This work was carried out in accordance with the guidelines of the Faculty of Life Sciences at University of Benin for animal use.

A total of 32 rats were randomly assigned into two groups, normal 8 rats and obese 24 rats. Obesity was

induced in the 24 rats by feeding with high fat diet (HFD) for 14 weeks as indicated in Table 1 below. Rats fed with HFD whose body weights were significantly increased compared to the control were

Composition	Food /Supplements	NPD (%)	HFD (%)
Carbohydrates	Garri	60.0	25.0
Fat	Butter	5.0	50.0
Proteins	Bonga Fish	30.0	20.0

Experimental Design and Animal Grouping

After induction of obesity, the rats were included in four groups and 8 rats per group. Group 1 was fed the NPD and water *ad libitum* for 18 weeks. Group 2-4 were fed HFD and water *ad libitum* for 14 weeks, thereafter received treatment as shown in Table 2 for 4

Table 2: Experimental design and animal grouping

Group	Nutrition for 14 weeks	Treatment for 4 weeks
1	Normal Pellet Diet (NPD)	Normal Control
2	High Fat Diet (HFD)	HFD Obese Control
3		500 mg/kg b.w *A. boonei*
4		500 mg/kg b.w *S. jollyanum*

Body weight and BMI determinations

The body weight of the rats was measured weekly in grams (g). The body length (nose-to-anus) was determined in all rats. The measurement was made using a ruler marked in centimetres (cm). The body weight and body length will be used to determine the Body mass index (BMI).

- Body mass index (BMI) = body weight $(g)/length^2$ (cm^2)

Biochemical Investigations

Blood was collected from fasting rats by cardiac puncture, allowed to clot and serum separated and used to assay for the following parameters by the methods indicated in parenthesis: creatinine (Bartels and Bohmer, 1972), urea (Weatherburn, 1967), uric acid (Fossati *et al.*, 1980), alanine aminotransferase (Reitman and Frankel, 1957), aspartate aminotransferase (Reitman and Frankel, 1957), alkaline phosphatase (Rec. GSCC, 1972), creatine kinase (Szasz, 1975; Szasz, 1976) and lactate dehydrogenase (Klin, 1972). The rat liver was used to assay for reduced glutathione (Tietz, 1976), malondialdehyde (Ohkawa *et al.*, 1979), superoxide dismutase (Mistra and Fridovich, 1972) and catalase (Cohen *et al.*, 1970).

considered obese (Amin and Nagy, 2009; Mistry *et al.*, 2011).

Table 1: Composition of Experimental Diet

Fiber	Afrodak	1.5	1.5
Mineral mixture	Multi-minerals	2.5	2.5
Vitamin mixture	Multi-vitamins	1.0	1.0
Energy (KCal/g)		4.095	6.345

weeks. They had free access to their diets and water. The extracts were suspended in normal saline and then it was administered orally to the rats for 4 weeks using a gavage tube.

Statistical analysis

The experimental results were expressed as the Mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by One way ANOVA followed by Duncan's multiple range test. $P < 0.05$ was considered to be significant.

RESULTS

Phytochemical Screening

The bioactive compounds in the ethanolic stem bark extract of *A. boonei* and the ethanolic root extract of *S. jollyanum* are indicated in Table 3.

Table 3: Phytochemical constituents of the ethanolic extracts of the plants

S/No.	Phytochemical Constituents	*Alstonia boonei* (stem bark)	*Sphenocentrum jollyanum* (root)
1.	Alkaloid	+	+
2.	Saponin	+	+
3.	Tannin	+	+
4.	Steroid	+	-
5.	Flavonoid	+	+
6.	Cardiac glycosides	+	+
7.	Terpenoids	+	+
8.	Reducing sugar	-	+

(+) = Presence,   (-) = Absent

Acute Toxicity (LD$_{50}$) of Plants

General weakness and sluggishness were the major behavioral changes observed in the rats at 6400 mg/kg b. wt. oral dose of *A. boonei* and *S. jollyanum*. These behavioral changes disappeared after 1 hour of observation. No death was recorded at any of the doses administered. Therefore, oral LD$_{50}$ of *A. boonei* and *S. jollyanum* was determined as $\geq$ 6400 mg/kg b. w. in wistar rats.

Duration of Obesity Treatment of the Rats
Table 4: Body weight of rats treated with the plant extracts

	Body weight (g)				
GROUP	WEEK 14	WEEK 15	WEEK 16	WEEK 17	WEEK 18
Normal Control	270.33 ± 6.39[b]	274.00 ± 4.93[b]	275.67 ± 8.09[b]	290.33 ± 8.41[b]	293.67 ± 9.17[c]
HFD Obese Control	351.33 ± 4.67[a]	364.67 ± 9.02[a]	374.67 ± 5.81[a]	387.67 ± 8.29[a]	395.00 ± 6.35[a]
HFD + *A. boonei*	356.67 ± 3.38[a]	356.33 ± 4.70[a]	358.00 ± 9.54[a]	365.00 ± 7.57[a]	369.00 ± 6.25[b]
HFD + *S. jollyanum*	353.67 ± 8.29[a]	355.00 ± 10.79[a]	367.33 ± 10.68[a]	381.67 ± 8.67[a]	379.67 ± 9.77[ab]

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different (p < 0.05).

The body weight of the HFD obese control group, maintained significantly increased body weight compared to the normal control throughout the treatment period. In the HFD groups, the groups treated with *A. boonei* had significant decrease in body weight compared to the HFD control at the 18th week. Though, there was weight reduction in the group treated with *S. jollyanum*, the decrease was not significant (Table 4).

Table 5: BMI of rats treated with the plant extracts

	BMI (g/cm^2)				
GROUP	WEEK 14	WEEK 15	WEEK 16	WEEK 17	WEEK 18
Normal Control	0.74 ± 0.05[c]	0.71 ± 0.02[c]	0.66 ± 0.01[c]	0.66 ± 0.01[c]	0.64 ± 0.00[c]
HFD Obese Control	1.04 ± 0.03[a]	1.05 ± 0.05[a]	1.02 ± 0.04[a]	0.99 ± 0.04[a]	0.92 ± 0.04[a]
HFD + *A. boonei*	0.92 ± 0.03[b]	0.87 ± 0.03[b]	0.83 ± 0.01[b]	0.82 ± 0.02[b]	0.80 ± 0.02[b]
HFD + *S. jollyanum*	1.03 ± 0.03[a]	0.94 ± 0.03[b]	0.87 ± 0.02[b]	0.88 ± 0.02[b]	0.83 ± 0.02[b]

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different (p < 0.05).

The BMI of the HFD obese control group remained significantly increased compared to the normal control at the end of the treatment. The BMI of the groups treated with *A. boonei* and *S. jollyanum* were significantly decreased compared to the HFD control (Table 5).

Table 6: Kidney function assessment of rats treated with the plant extracts

GROUP	CREATININE (mg/dl)	UREA (mg/dl)	URIC ACID (mg/dl)
Normal Control	1.33 ± 0.13[b]	17.75 ± 0.31[b]	9.52 ± 0.73[b]
HFD Obese Control	3.07 ± 0.13[a]	33.31 ± 1.74[a]	16.62 ± 0.48[a]
HFD + *A. boonei*	2.67 ± 0.23[a]	18.40 ± 0.86[b]	10.67 ± 0.79[b]
HFD + *S. jollyanum*	3.07 ± 0.13[a]	33.81 ± 1.38[a]	14.17 ± 0.91[a]

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different (p < 0.05).

Serum creatinine, urea and uric acid concentrations were significantly higher in the HFD obese control group compared to the normal control. In the HFD groups, the urea and uric acid levels were significantly decreased in *A. boonei* treated rats, but not in rats treated with *S. jollyanum* (Table 6).

Table 7: Assessment of Liver function in rats treated with the plant extracts

GROUP	ALT (U/l)	AST (U/l)	ALP (U/l)
Normal Control	4.18 ± 0.36[b]	6.92 ± 0.46[b]	2.77 ± 0.04[d]
HFD Obese Control	4.93 ± 0.14[a]	9.45 ± 0.33[a]	13.39 ± 0.97[a]
HFD + *A. boonei*	3.05 ± 0.09[c]	4.17 ± 0.09[c]	7.90 ± 0.21[b]
HFD + *S. jollyanum*	3.55 ± 0.38[c]	6.80 ± 0.36[b]	4.72 ± 0.57[c]

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different (p < 0.05).

The ALT, AST and ALP levels were significantly elevated in the HFD obese control group compared to the normal control. *A. boonei* treated group had significantly decreased ALT and AST compared to the normal control while *S. jollyanum* significantly decreased ALT compared to the normal control, but

showed no significant difference in the AST level. However, *A. boonei* and *S. jollyanum* decreased ALP levels when compared to the HFD obese control group but not to the normal control. (Table 7).

Table 8: Lipid peroxidation and antioxidant enzymes of rats treated with the plant extracts

GROUP	MDA (U/100mg)	CATALASE (K)	SOD (U/100mg)	GSH (mM)
Normal Control	23.92 ± 0.78^c	158.89 ± 4.37^a	167.40 ± 5.78^a	110.33 ± 2.60^a
HFD Obese Control	28.34 ± 0.81^a	142.87 ± 1.65^b	71.50 ± 0.64^d	32.67 ± 1.45^c
HFD + *A. boonei*	22.04 ± 0.66^{ab}	162.96 ± 5.05^a	104.00 ± 6.58^c	50.00 ± 0.58^b
HFD + *S. jollyanum*	25.69 ± 0.64^{ab}	169.37 ± 2.55^a	135.97 ± 2.7^b	46.00 ± 2.31^b

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

The hepatic MDA was significantly high, while hepatic catalase, SOD and GSH were significantly lower in the HFD obese control group compared to the normal control. Both *A. boonei* and *S. jollyanum* treated groups showed significant increase in catalase, SOD and GSH activity and no significant decrease in MDA (Table 8).

Table 9: CK and LDH activities in rats treated with the plant extracts

GROUP	CK Activity (U/l)	LDH (U/l)
Normal Control	32.23 ± 1.91^c	149.61 ± 5.25^c
HFD Obese Control	73.02 ± 2.96^a	213.73 ± 14.14^{ab}
HFD + *A. boonei*	32.40 ± 1.22^c	149.61 ± 10.69^c
HFD + *S. jollyanum*	38.52 ± 3.07^{bc}	181.67 ± 14.14^{bc}

Values are expressed as means ± SEM. Means in the same column not sharing common letter(s) are significantly different ($p < 0.05$).

The CK-activity and LDH level were significantly elevated in the HFD obese control group compared to the normal control. Among the HFD treated groups, *A. boonei* treated group had significantly reduced CK and LDH activity, whereas only the CK activity was significantly reduced by treatment with *S. jollyanum* (Table 9).

Discussion and Conclusion

Obesity is currently of serious public health concern globally, particularly in the economically advanced nations and rising in the developing countries. A lot of efforts to mitigate the problem have called for different investigations using various materials including plants as seen in this study. The presence of alkaloid, saponin, tannin, steroid and flavonoid in the stem bark ethanolic extracts of *A. boonei* (Table 3) is consistent with the earlier works (Akinmoladun *et al.*, 2007; Amole and Ilori, 2010; Onyeneke and Anyanwu, 2013). The phytochemical constituents of the ethanolic root extract of *S. jollyanum* showed the presence of alkaloid, saponin, tannin, flavonoid, cardiac glycoside, terpenoid and reducing sugars. This is in tandem with the earlier study by Mbaka *et al.* (2009).

A synergistic relationship amongst phytochemicals has been adduced to be responsible for the overall beneficial effect derivable from plants (Liu, 2004). Cardiac steroids and flavonoids lower the risk of heart diseases (Okwu and Okwu, 2004), a common risk factor of obesity. The decrease in CK and LDH activity by *A. boonei* and *S. jollyanum* may be due to the presence of flavonoids. Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative damage (Okwu, 2004).

In laboratory animals, obesity has been described as significant increase in body weight (Amin and Nagy, 2009) and/or increase in BMI (Novelli *et al.*, 2007). The high fat diet used in this study which contained 60% fat induced obesity in the rats. This is consistent with other reported works were obesity has been induced in rats with hypercaloric diets compounded by adding more fats (Vinicius *et al.*, 2006; Gajda, 2009). *A. boonei* decreased the body weight and BMI of the rats. Similarly, it has been reported in a toxicity study, that *A. boonei* decreased significantly the body weights of rats treated for 4 and 12 weeks (Raji *et al.*, 2005).

In another toxicity study, *S. jollyanum* (root) exhibited dose dependent increase in body weight that was comparably higher than the control group (Mbaka and Adeyemi, 2010). Again, there was significant increase in weight of the rats after 120 days of seed extract administration of *S. jollyanum* with no

abnormal gross changes (Mbaka and Owolabi, 2011). These results are inconsistent to our findings which showed that *S. jollyanum* decreased body weight (although not significantly) and BMI of the rats after treatment for 4 weeks.

ALT and AST are enzymes known to be reliable indices for hepatotoxicity assessment (Hayes, 1989). Increased ALT and AST levels have been related with hepatic fat deposition which leads to obesity (Wallace *et al.,* 2007; Choi, 2003; Amin and Nagy, 2009). *A. boonei* and *S. jollyanum* decreased ALT and AST activity thereby enhancing the metabolism of fat.

The HFD and HCD obese rats showed a high significant increase in the concentration of serum creatinine, urea and uric acid, compared with the normal control (Table 7), suggesting renal impairment a situation familiar in obesity, which is in agreement with the results of Cindik *et al*. (2005) and Amin and Nagy (2009)**.** Creatinine and urea, which are non-protein nitrogenous substances and end products of protein metabolism that must be removed continually (Guyton, 1981). Urea levels may be elevated (a condition called azotemia) in both acute and chronic renal (kidney) impairment. Obesity has been implicated as a risk factor for nephrolithiasis, a kidney stone disease (Taylor *et al.,* 2005).

Uric acid is normally excreted in small amounts in the urine of humans. Uric acid concentrations may become elevated in kidney diseases and leukemia. Weight reduction has been associated with modest lowering of serum uric acid (Choi *et al.,* 2005). In this present study, only the HFD group treated with *A. boonei* reduced significantly the urea and uric acid levels in the treated groups. The creatinine, urea and uric acid levels were not significantly altered by *S. jollyanum* compared to the HFD obese control. This follows the result by Mbaka and Adeyemi (2010) in a chronic toxicity test, they suggested that the insignificant changes in serum creatinine and urea levels may be due to the relatively non-nephrotoxic effect of the extract suggesting that the activity of protein metabolism may be maintained within the normal range.

Increased oxidative stress is a common phenomenon in obesity. This is revealed by a significant increase in the levels of MDA and a significant decrease in hepatic CAT, SOD and GSH. CAT, SOD and GSH are antioxidants that are expected to counter the effects of free radicals produced by oxidative stress. Numerous oxygenated compounds, mainly aldehydes such as malondialdehyde (MDA), are produced during the attack of free radicals on membrane lipoproteins and polyunsaturated fatty acids (PUFA). Hyperglycaemia in the HFD group activates different pathways leading to increased oxidative stress. Increased activity of the polylol pathway inhibition of the pentose phosphate pathway as a result of hyperglycaemia resulted in decreased intracellular levels of NADPH, which is required for regeneration of GSH from its oxidized form GSSG (Brownlee, 2001). The net result was increased levels of cellular superoxides radicals, hydrogen peroxides, hydroxyl radicals as well as other radicals.

In addition oxidative stress may be increased in metabolic syndrome such as obesity, due to dyslipidemia resulting from increased levels of FFA and TGs that leads to increased formation of foam cells, rendering LDL less dense and more vulnerable to oxidation and uptake by macrophages (Holvoet, 2008). Both *A. boonei* and *S. jollyanum* showed significant increase in CAT, SOD and GSH activity and no significant decrease in MDA in the HFD group. The ability for *A. boonei* and *S. jollyanum* to increase antioxidant enzymes activity could be attributed to the presence of flavonoids in these plants. This shows that flavonoids play a vital biological role as antioxidants, including the function of scavenging reactive oxygen species (Pietta and Simonetti, 1998).

The control of the obese rats showed a significant increase in the activity of CK-NAC and LDH when compared to the normal rats, which is in agreement to that reported by Diniz *et al*. (2008) and Amin and Nagy (2009). Persistent increase in CK-NAC and LDH activities might be signs of the development of heart disease, one of the risk factors of obesity. Treatment with *A. boonei* produced a significant decrease in the activity of CK-NAC and LDH.

Conclusion

The ethanolic extracts of *A. boonei* and *S. jollyanum* have shown varying potentials to reduce weight and to improve the function of organs by reducing oxidative stress and fat deposition in the liver of rats fed high fat diets. However, the ethanolic extracts of *A. boonei* stem bark showed more promising antiobesity potential than that of *S. jollyanum* in rats fed high fat diets.

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