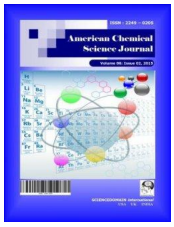

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Antioxidant Potentials of L-Ascorbic Acid (L-AA) and Butylated Hydroxytoluene (BHT) in CCl_4-Induced Oxidative Damage of Soft Tissues in Wistar Rats

O. M. Ighodaro[1*], J. O. Omole[1], A. O. Aminu[1], A. M. Adeosun[1] and A. I. Ogunlana[1]

[1]Department of Biochemistry, Lead City University, Ibadan, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OMI designed the study, wrote the protocol and wrote the first and final drafts of the manuscript. Authors AOA and AIO managed the analyses of the study. Authors AMA and JOO managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The antioxidant potentials of Butylated hydroxytoluene (BHT) and L-Ascorbic acid (L-AA) in CCl_4-induced tissue damage were compared in adult female Wistar rats. Toxicity was induced in the animals via a single dose (i.p) of 20% 2 mL/Kg body weight (BW) of CCl_4. BHT and L-ascorbic acid, each at a dose of 100 mg/kg BW were orally administered (twice daily for 7 days) to different groups of CCl_4-treated animals. Biochemical analyses were carried out on the supernatant fractions of the liver and kidney homogenates to estimate the levels of tissue protein and malondialdehyde (MDA)

**Corresponding author: E-mail: macigho@yahoo.com;*

as well as the activities of antioxidant enzymes: gluthatione s-transferase (GST), superoxide dismutase (SOD), and catalase (CAT). The data obtained showed that the evaluated antioxidants (BHT and L-ascorbic acid) elicited notable ameliorative effects against CCl_4-induced tissue damage. Comparatively, BHT elicited relative higher catalase activity and decreased MDA concentration in CCl_4-induced liver and kidney damage in rats. BHT also produced significantly higher protective effect in terms of SOD activity in the kidney of rats. Conversely, L-Ascorbic acid showed a relative higher GST and SOD activities in CCl_4 induced liver damage alone. There was no significant difference in the effect of the two antioxidants on the activity of GST in the kidney. Overall, the data of this study appear to score BHT (a synthetic antioxidant) relatively higher than ascorbic acid (a natural antioxidant) in terms of *in vivo* antioxidant capacities within the limit of the parameters assessed. It also demonstrates that BHT elicits its antioxidant potential against CCl_4 induced reactive species mainly through promotion of catalase activity and inhibition of lipid peroxidation whereas ascorbic acid maximizes its antioxidant effects against the same toxicant by promoting GST and SOD activities particularly in the liver.

Keywords: Natural antioxidants; synthetic antioxidants; carbon tetrachloride; tissue damage.

1. INTRODUCTION

Production of free reactive species by CCl_4 metabolism in tissues such as liver and kidney has been well documented and evidently associated with increased lipid peroxidation and oxidative stress, leading to tissue damage [1-4]. Also, the protective function of antioxidants via cascades of reactions against oxidative damage of biomolecules such as lipids, nucleic acids and proteins in tissues by free radicals or reactive species is popular [5-7].

L-ascorbic acid (L-AA), a natural antioxidant fulfils essential antioxidant and metabolic functions in the life of animals and plants. It readily scavenges reactive oxygen and nitrogen species, such as superoxide and hydroperoxyl radicals, singlet oxygen, peroxynitrite, nitroxide radicals, and hypochlorous acid, thus effectively protecting other substrates from oxidative damage [8,9].

Besides, there are a number of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ). They are extensively used as food additives to increase the shelf life, especially of lipids and lipid-containing products, by retarding the process of lipid peroxidation in order to prevent food deterioration during processing and storage [10]. Study on the effect of butylated hydroxytoluene and butylated hydroxyanisole on CCl_4 induced-hepatotoxicity in rats by Elgazar et al. [11] revealed that BHA and BHT significantly decrease elevated serum level of liver enzymes and malondialdehyde (a biomarker for lipid peroxidation) with significant increase in activities of gluthathione peroxidise (GPx), superoxide dismutase (SOD), and catalase (CAT). However, there is paucity of information on the comparative potency of synthetic antioxidants and naturally occurring antioxidants. More so, the role of natural antioxidants in combating free radicals is apparently favoured compared to synthetic antioxidants. This observation underscores the need to comparatively assess the antioxidant potentials of both class of antioxidants (natural and synthetic). The current study therefore compares the effects of butylated Hydroxytoluene (BHT) (a synthetic antioxidant) and L-ascorbic acid (a naturally abundant antioxidant) on CCl_4-induced tissue damage (liver and kidney) in animal model.

2. MATERIALS AND METHODS

2.1 Experimental Design and Animal Treatment

The animal procedure was carried out according to the standard protocols approved by Animal Research Ethics Committee of Faculty of Information Technology and Applied Sciences, Lead City University, Ibadan Nigeria, for the use of experimental animals in research. Twenty female adult Wistar rats (mean weight of 139.2±12.3) were used for the study; and were purchased from a local breeder. The animals were handled humanely, kept in metallic suspended cages in a well ventilated and hygienic rat house under standard conditions of temperature and humidity. They were allowed to acclimatize for two weeks and maintained on normal laboratory chow (Vital feeds) and with water *ad libitum*. They were

subjected to natural photoperiod of 12 hours light and 12 hours dark cycle. All animal experiments were carried out without anaesthesia during the study.

The animals were randomly assigned to four (4) groups with five animals in each group (n=5). Oxidative damage was induced in experimental animals by injecting intraperitoneally with a single dose of 20% 2 mL/kg BW of carbon tetrachloride dissolved in olive oil. 100 mg of Butylated hydroxytoluene (BHT) and 100 mg Ascorbic acid per kilogram body weight were used to challenge the induced oxidative damage, and were orally administered to the animals, twice daily using intubators for a period of 7 days.

Group A: Rats treated with olive oil (0.5 mL), **(Control)**
Group B: Rats treated intraperitoneally with CCl_4 **(CCl_4 alone)**
Group C: CCl_4 and Butylated hydroxytoluene (BHT) treated rats **(CCl_4 + BHT)**
Group D: CCl_4 and Ascorbic acid treated rats **(CCl_4 + LAA)**

All animals were allowed equal access to normal laboratory chow and water *adlibitum.*

2.2 Tissue Preparation and Biochemical Analysis

At the end of 7 days of administration, the rats were sacrificed by cervical dislocation and the target organs (liver and kidney) were harvested and homogenised with ice cold Tris-HCL buffer (0.1 M, pH 7.4) using a Potter Elvehjem type homogenizer. The homogenization was done using 2 ml buffer to 1 g of liver and 3 ml buffer to 1 g of kidney. The homogenate was centrifuged at 4°C at 5500 rpm for 10 minutes using 3000i centrifuge and the resultant supernatant was used for different biochemical assays.

Protein concentrations of post mitochondrial-fractions of different organs were estimated by the procedure of Lowry et al. [12] using bovine serum albumin (BSA) as standard. Catalase activity was determined according to the method of Sinha [13]. The cytosolic glutathione–S–transferase activity was determined spectrophotometrically at 37°C according to the procedure of Habig et al. [14]. The levels of total SOD activty in liver and kidney homogenates were determined by the method of Misra and Fridovich [15]. A breakdown product of lipid peroxidation thiobarbituric acid reactive substance (TBARS) was measured by the method of Buege and Aust [16].

3. RESULTS

Table 1 shows the difference in mean weight of rats. Comparisons were made between the initial and final body weights of rats in each group. During the experiment, there was a decrease (5.4%) in the body weights of rats that were treated with CCl_4 alone. However, rats in other experimental groups had increased body weight, with the highest increase (8.1%) observed in the control animals. Treatment of rats with ascorbic acid following CCl_4 administration caused a significant ($P<0.05$) increase in weight gain (5.5%) compared to the use of BHT which resulted in 2.1% gain in body weight of rats.

The effects of the different treatments on the relative organ weights are shown in Fig. 1. The relative weight of the liver and kidney of rats treated with CCl_4 alone when compared to control group decreased significantly by 26.7 and 26.2% respectively. Co-treatment of rats with BHT or Ascorbic acid markedly reduced liver weight loss to 8.04% when compared to control animals. In the case of kidney, co-treatment with BHT reduced weight loss from 26.2% to 7.14 whereas co-treatment with ascorbic acid reduced weight loss to 11.90%.

Table 1. Effects of BHT and ascorbic acid on body weight of CCl_4 - treated rats

Experimental group	Final body weight (g)	Initial body weight (g)	Change in body weight (g)	% Δ in body weight
Control	169.5±0.0	157.0±0.0	+12.68±0.5	8.1
CCl_4 alone	134.0±0.0	141.0±0.0	-7.57±0.5[a]	5.4
CCl_4 +BHT	136.0±0.0	129.0±0.0	+7.14±0.4	5.5
CCl_4 + Ascorbic acid	130.0±0.0	127.8±0.0	+2.60±0.2[b,c]	2.1

Values are expressed as means±SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05
b=significant when compared to CCl_4 alone at 95%CI, P<0.05
c=significant when compared to CCl_4+BHT or CCL_4+Ascorbic acid at 95%CI, P<0.05

The liver and kidney protein concentrations of the different group of rats are shown in Fig. 2. Intraperitoneal administration of CCl_4 was observed to cause a significant reduction in liver protein by 11.76% relative to control group. It however did not significantly affect the level of protein in the kidney (0.22% decrease). Co-treatment of rats with BHT as well as with ascorbic acid did not cause any significant difference in kidney protein. Ascorbic acid administration significantly increased liver protein compared to control group and BHT co-treated group.

The SOD activities in the liver and kidney of rats treated with CCl_4 alone when compared to control group decreased significantly by 91.95 and 32.52% respectively (Table 2). Co-treatment of rats with BHT markedly restored liver and kidney SOD activities by 80.28 and 46.79% respectively when compared to CCl_4 group. Conversly, Co-treatment of rats with Ascorbic acid caused a significant elevation in liver and kidney SOD activities by 92.04 and 34.64% respectively when compared to CCl_4 group (Table 2).

BHT caused a significantly higher SOD activity in the kidney compared to Ascorbic acid. Whereas higher SOD activity in the liver was observed in animals co-treated with Ascorbic acid.

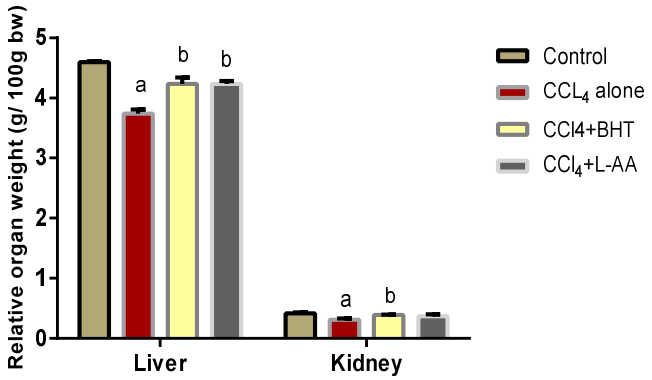

Fig. 1. Effects of BHT and ascorbic acid on relative organ weight of CCl_4 - treated rats

Values are expressed as means ± SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05 b=significant when compared to CCl_4 alone at 95%CI, P<0.05

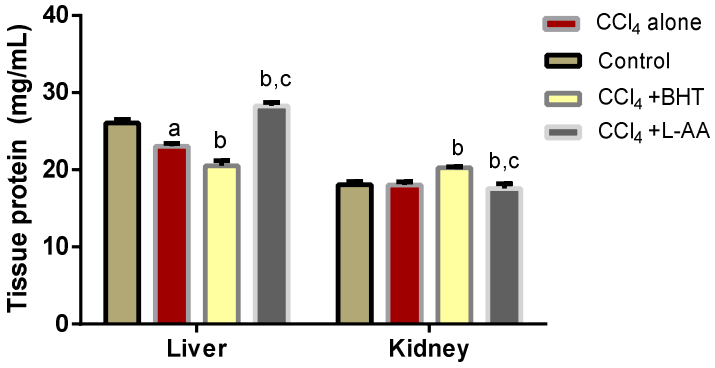

Fig. 2. Effects of BHT and ascorbic acid on tissue protein of CCl_4 - treated rats

Values are expressed as means ± SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05 b=significant when compared to CCl_4 alone at 95%CI, P<0.05 c=significant when compared to CCl_4+BHT at 95%CI, P<0.05

Table 3 shows the effects of treatments on GST activity rats. GST activity was significantly reduced by 47.92 and 80.28% respectively in the liver and kidney of rats that were administered CCl_4 alone compared to control group.

Co-treatment of rats with BHT markedly increased GST activities in the liver and kidney by 80.28 and 46.79% respectively when compared to CCl_4 group.

Conversely, co-treatment of rats with Ascorbic acid markedly restored liver and kidney GST activities by 94.03 and 45.50% respectively when compared to CCl_4 group.

Ascorbic acid caused a significantly higher GST activity in the liver of rats compared to BHT, whereas no significant difference in kidney GST activity was observed in respect to both antioxidants (BHT and Ascorbic acid).

MDA levels in the liver and kidney of rats treated with CCl_4 alone were significantly elevated by 42.23 and 39.52% as compared to control group. Co-treatment of rats with BHT markedly lowered liver and kidney MDA by 20.23 and 32.86% respectively when compared to CCl_4 group. Conversely, Co-treatment of rats with Ascorbic acid caused a significant reduction in liver and kidney MDA by 11.17 and 11.34% respectively when compared to CCl_4 group (Fig. 3). The lowering effect of BHT on CCl_4-induced MDA level was significantly higher in both the liver and kidney of rats compared to Ascorbic acid.

Catalase activity was significantly reduced by 36.17 and 27.30% respectively in the liver and kidney of rats that were administered CCl_4 alone compared to control group. Co-treatment of rats with BHT markedly increased catalase activities in the liver and kidney by 23.07 and 20.77% respectively when compared to CCl_4 group.

On the other hand, co-treatment of rats with Ascorbic acid markedly restored liver and kidney catalase activities by 20.00 and 18.66% respectively when compared to CCl_4 group (Table 4).

There was no significant difference in the effects of both BHT and Ascorbic acid on catalase activities in the liver as well as kidney of rats.

4. DISCUSSION

The protective role of antioxidants via cascades of reactions against oxidative damage of biomolecules such as lipids, nucleic acids and proteins in tissues by free radicals or reactive species is well documented [2-7].

Table 2. Effects of BHT and ascorbic acid on SOD activity in CCl_4 - treated rats

Experimental group	Liver SOD (U/mg protein)	Kidney SOD (U/mg protein)
Control	1.74±0.03	1.23±0.07
CCl_4 alone	0.14±0.02[a]	0.83±0.02[a]
CCl_4 +BHT	0.71±0.02[b]	1.56±0.01[b]
CCl_4 + Ascorbic acid	1.76±0.03[b,c]	1.27±0.11[b,c]

Values are expressed as means ± SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05 b=significant when compared to CCl_4 alone at 95%CI, P<0.05 c=significant when compared to CCl_4+BHT at 95%CI, P<0.05

Table 3. Effects of BHT and ascorbic acid on Glutathione s-transferase activity in tissues of CCl_4 - treated rats

Experimental group	Liver GST (ug/min/mg protein)	Kidney GST (ug/min/mg protein)
Control	2.17±0.13	5.53±1.32
CCl_4 alone	1.13±0.05[a]	1.09±0.04[a]
CCl_4 +BHT	1.52±0.05[b]	2.05±0.05[b]
CCl_4 + Ascorbic acid	2.18±0.11[b,c]	2.00±0.08[b]

Values are expressed as means ± SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05 b=significant when compared to CCl_4 alone at 95%CI, P<0.05 c=significant when compared to CCl_4+BHT at 95%CI, P<0.05

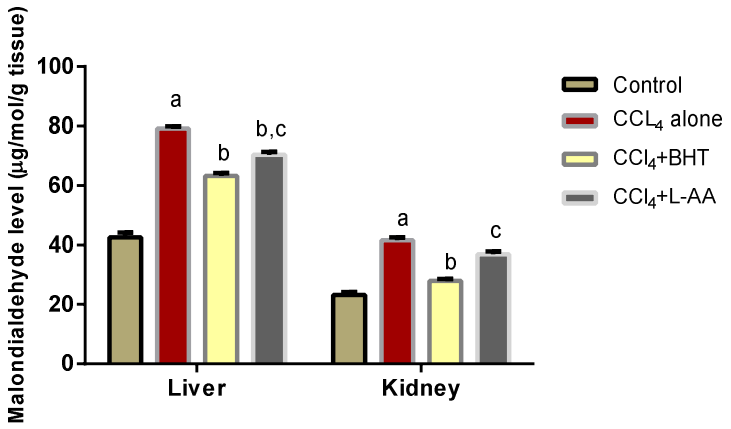

Fig. 3. Effects of BHT and ascorbic acid on MDA level in tissues of CCl_4 - treated rats

Values are expressed as means ± SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05+ b=significant when compared to CCl_4 alone at 95%CI, P<0.05 c=significant when compared to CCl_4+BHT at 95%CI, P<0.05

Table 4. Effects of BHT and ascorbic acid on catalase activity in tissues of CCl_4 - treated rats

Experimental group	Liver catalase (Kat f)	Kidney catalase (Kat f)
Control	23.50±1.71	21.00 ± 1.58
CCl_4 alone	15.00 ± 1.08[a]	15.25 ± 1.25[a]
CCl_4 +BHT	19.50 ± 0.65[b]	19.25 ± 0.48[b]
CCl_4 + Ascorbic acid	18.75 ± 0.85[b]	18.75 ± 1.32

Values are expressed as means ± SEM of 5 rats. The statistical significance of difference between groups were analyzed using the one-way analysis of variance (ANOVA) followed by independent-sample t test. Statistical test were performed using prism graphpad, version 6.0 a=significant when compared to control at 95% CI, P<0.05+ b=significant when compared to CCl_4 alone at 95%CI, P<0.05

In accordance, the result of the current study revealed that the evaluated antioxidants (BHT and Ascorbic acid) elicited notable combative or ameliorative effects against CCl_4-induced cellular oxidative damage by significantly replenishing the body and organ weights, the protein level and antioxidant enzyme activities altered by CCl_4 in the tissues of rats.

However, the cardinal focus of this study was to compare the effects of BHT, a synthetic anti-oxidant and L-ascorbic acid, a naturally occuring antioxidant against CCl_4 -induced oxidative damage in soft tissues of Wistar rats.

The administration of CCl_4 caused significant reduction in body and organ (liver and kidney) weights of rats. This is arguably due to the toxicity associated with CCl_4 metabolism which probably resulted in lowered appetite or impairment of nutrient absorption following the damaging of cell linings of the stomach and of the intestine. The decrease in the body and organ weights of rats following CCl_4 treatment observed in this study is consistent with previous reports on other free radical-generating compounds such as Di-n-Butylphthalate and ethanol [17,18].

Concomitant administration of the antioxidants (BHT and ascorbic acid) significantly enhanced organ and body weight gain when compared to rats treated with CCl_4 alone, but significantly lower as compared with control animals. Moreover, rats co-treated with ascorbic acid showed more body weight gain (5.5%) as compared with those administered BHT (2.1%). Meanwhile no significant difference was observed in the effects of both antioxidants on organ weights. Besides its antioxidant status, the role of ascorbic acid in the synthesis of some

structural proteins such as collagen is well established. This may have contributed to the relatively higher body weight gain associated with ascorbic acid. More so, ascorbic acid is a notable food nutrient (vitamin) required for body growth and repair.

CCl_4 generates a highly reactive species which among other things lower tissue protein, deplete enzymatic antioxidant defenses (SOD, CAT, GST, GPX, GR) and attack lipid molecules to initiate lipid peroxidation of cell membranes [19]. The data obtained in the present study corroborate the above claim.

Conversely, treatment of rats with BHT or ascorbic acid at a dose of 100 mg/kg of body weight following CCl_4 administration (20% 2 mL/kg of body weight) resulted in significant ($P<0.05$) increase in liver protein and antioxidant enzymes activities as well as significant decline in MDA concentrations in the liver and kidney of rats when compared to rats treated with CCl_4 alone. Surprisingly, both CCl_4 and the antioxidants had no significant effect on kidney protein.

Glutathione-S-transferase (GST) performs a significant function in the detoxification and metabolism of many xenobiotics and endobiotics substances which have hydrophilic functional groups [20]. On the other hand, super oxide dismutase catalyses the dismutation of superoxide anion radicals into hydrogen peroxide (H2O2) [21]. In this study SOD and GST activities were found to be significantly higher in the liver of rats treated with ascorbic acid following CCl_4 administration when compared to those that were administered BHT. There was no significant difference in the effects of both antioxidants on the kidney GST activity. However, BHT produced a significantly better restorative effect on SOD activity in the kidney of rats.

Catalase is an essential and integral component of the cellular antioxidant defense system. It catalyses the breakdown of hydrogen peroxide which results from normal body metabolism and SOD activity [22]. The result obtained in the present study showed that catalase activity significantly increased in the liver and kidney of rats treated with BHT following CCl_4 administration as compared with those that were administered ascorbic acid. The observed increase in CAT activity is due to the accumulation of hydrogen peroxide following SOD activity.

High level of Malondialdehyde (MDA) is an index or biomaker of lipid peroxidation. The higher the MDA level in tissues, the more the degree of lipid peroxidation [21]. In the current study, administration of CCl_4 produce marked increase in the level of MDA in the liver and kidney of rats. Both antioxidants (Ascorbic acid and BHT) reduced the level of MDA caused by CCl_4 – oxidative damage in tissue of rats.

Notably, the MDA concentrations in both the liver and kidney of rats treated with BHT following CCl_4 administration was significantly reduced as compared to the concentrations recorded in rats treated with ascorbic acid.

The relative higher catalase activities and decreased MDA concentration elicited by BHT in CCl_4 induced liver and kidney damage rats adduce better antioxidant effect to the compound compared to ascorbic acid in respect to their ability to protect against CCl_4-induced oxidative damage via the induction of catalase activity and inhibition of lipid peroxidation of membranes.

Conversely, data of the study showed that ascorbic acid elicited a relative higher GST and SOD activities in CCl_4 induced liver damage. This connotes that the molecule has better antioxidant effect compares to BHT vis-a-vis GST and SOD activities in the liver of rats. However, BHT produced significantly higher protective effect in terms of SOD and GST activities in the kidney of rats.

5. CONCLUSION

Overall, the data of this study appear to score BHT (a synthetic antioxidant) relatively higher than ascorbic acid (a natural antioxidant) in terms of their *in vivo* antioxidants capacities within the limit of the parameters assessed. It also demonstrates that BHT elicits its antioxidant potential against CCl_4- induced reactive species mainly through promotion of catalase activity and inhibition of lipid peroxidation whereas ascorbic acid maximizes its antioxidant effects against the same toxicant by promoting GST and SOD activities particularly in the liver.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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