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Protective Effects of Trans-Ferulic acid on Tamoxifen Induced Heptatic and Renal Oxidative Stress in Male Wister Albino Rat

[*1]OGBUTA, AA; [1]GIVEN, OT; [2]EBINISTEI, P; [2]ALEXIS, EO; [1]WELLINGTON, OE; [2]TEKE, EC; [2]MANDA, AE

*[*1]Department of Chemical Sciences, [2]Department Biological Sciences University of Africa, Toru--Orua, Bayelsa State, Nigeria.*

**Corresponding author Email: ogbutaanthony@yahoo.com*
Co-Authors Email: obee.gc@gmail.com; precious@gmail.com; okabsmed@gmail.com; teke.calmday@uat.edu.ng; ewellington41@yahoo.com; manda.alice@uat.edu.ng

ABSTRACT: This study was designed to assess the protective effects of trans-ferulic acid on tamoxifen induced hepatic and renal oxidative stress in male Wister albino rates using appropriate standard methods. Twenty four male albino rats were randomly divided into 4 (four) groups of 6 (six) animals each. Group A, B, C and D where group A is control animals group B and C animals were treated with 0.4 mg/kgof tamoxifen and100 mg/kg body weight of trans-ferulic acid respectively. Group D animals were simultaneously administered with 100 mg/kg of trans-ferulic acid and 0.4 mg/kg of tamoxifen for twenty one days. UV/VIS spectrophotometer was used for the analysis of indicators. A significant elevation was observed in plasma bilirubin, urea and creatinine by tamoxifen treated group with mean concentrations of 35.50 ± 1.8 mg/dl, 2.15±0.06 mg/dl and 7.80 ±9.29 mg/dl respectively, when observed with mean concentrations of control group 14.7±0.31 mg/dl, 0.98±0.05 mg/dl and 3.31±0.15 mg/dl. While the mean concentrations of trans-ferulicacid treatment were 21.2±0.36 mg/dl,1.07±0.03 and 3.65 mg/dl, significantly protected on the effects of the tamoxifen- induced. ALP, AST, ALT activities were also increased significantly ($p<0.05$) on mean concentration of 65±1.0 U/L, 11.08±0.12 U/L and 678.20±13.30 U/L in the treated group by tamoxifen respectively when viewed with the control ($p<0.05$) and trans-ferulic acid treatment reduced the increased enzyme activities by mean concentrations of 43.6±1.0, 5.54±0.26 U/L and. Furthermore, tamoxifen administration decreased the level of hepatic superoxide dismutase (SOD), and catalase (CAT) activities by 32.5±2.30 µmole and 0.50±0.05µmole respectively when viewed with mean concentration of the control (49.0±1.15µmole and 1.37±0.02µmole) and trans-ferulic acid treatments significantly ameliorated the decreased in the hepatic enzymes activities ($P<0.05$) with mean valued 44.0±1.40 and 1.26 ± 0.05 µmole. Also the level of hepatic ascorbic acid was decreased significantly on treatment with tamoxifen by 41.7±2.9 U/L when compared with the mean concentration of the control (60.48±1.45 U/L), while treatment with trans-ferulic acid protected against the decreased level of ascorbic acid by 55.44±1.76 U/L. Moreover administration of tamoxifen caused a significant ($P<0.05$) increase in MDA by (97.9±6.9 U/L), while treatment with trans-ferulic acid protected against increased in the level of MDA to (22±0.70 U/L) .These study suggested that trans-ferulic acid reduced the effect of liver and kidney toxicity and induced stress posed by tamoxifen in rats.

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Keywords: Tamoxifen; Trans-Ferullic acid; Hepatic; Renal; Oxidative stress.

Tamoxifen is an anticancer drug that is used to stop or slow the growth of neoplasms. It travels through the body and kills cancerous cells, but during this process of eliminating the cancer, the anti-neoplastic agents posed some health risks to the patient receiving treatment because they killed healthy cells along with the cancerous ones (Goodman and Gilman, 2010). During the process of this drug metabolized in an organism's body system, certain metabolites (4-hydroxytamoxifen, 4-hydroxy-N-methyltamoxifen, and N-dimethyltamoxifen) accumulate in the liver and other tissues of the organism (Beguerie *et al.*, 2010).These compounds are builded up in an organism's body organs, thereby put the accumulated organs under a lot of stress (Yang *et al.*, 2013). It also prevents estrogen from connecting to its receptor by stopping the growth of cancer cells, just like a broken key in a lock prevents the use of any other key (Letrozole , 2009). Tamoxifen is widely used as a therapy for breast cancer because it is believed to be 65 % effective in treating tumors or tumorsstabiliza tion. However, it has been reported that the majority of patients treated with it eventually experienced health risks and that about 80 % of the patients with

metastatic cancer experienced stress and died from the disease (Thomas and Gustafsson, 2011). Transferulic acid is one of the most prevalent phenolic acids in plants and may be found in high concentrations in foods and fruits, it is one of the components of asafetida, the dried latex from the giant fernel (Ferula gigantea). This organic compound is found in coffee seeds, apple, peanut, orange, and as well as in seeds and cell walls of commelinid plants (such as cereals, oats, and pineapple).(Kumar *et al*., 2011). According to Okaba and Ebisntei, (2021) exposure of tramadol affected the biochemical and histopathological levels and spermatozoa production negatively in male Wister rat. Also, (Orororo et al., 2022) observed in their investigation that Hibiscus sabdariffal (Anthocyanins) was an effective antioxidant source that could eliminate free radicals. However the present study, will assed the protective effects of trans-ferulic acid on tamoxifen induced hepatic and renal oxidative stress in male Wister albino rates.

MATERIALS AND METHODS

Sample collection: Twenty four male wistar albino rats weighed between 140-165g were purchased from University animal house of Department of Chemical sciences of University of Africa Toru-Orua. Bayelsa State, Nigeria.Were used for the present study. They were kept in wired meshed cages with a good access to standard laboratory meal, tap water and temperature of (25-30 ^{o}C)and properly ventilated and artificial light up system of (12 hour dark cycle), care was taken to prevented the present of chemicals and contaminations in the room the animals were kept fed with commercial rat chow and water. All rats were acclimatized for three weeks before the onset of treatment.

Animal grouping and drug administration: The twenty four male albino rats were randomly grouped into four groups (6 animals per group) and properly labelled. (A, B, C, D) respectively.GroupA stands for (Control group) or untreated group, these animals were administered with distilled water for 21 days and B-group(were treated with Tamoxifen)(TMX), the animals were administered with (0.4 mg/kg body weight) of tamoxifen for 21 days while C- (TFA group): animals in this group were administered with (100 mg/kg body weight) of Trans-ferullic acid for 21 days and D- (TMX +TFA group): animals were administered with (0.4 mg/kg body weight) of tamoxifen and (100 mg/kg body weight) of trans-ferulic acid simultaneously for 21 days.The administration of the drugs occurred once daily, through oral gavage using oral intubated. All the animals were sacrificed 24- hours after the last administration

Samples preparation: Blood samples were collected through the retro-orbital plexus into heparinized tubes. The blood sample were centrifuged at 3000 rmp for 10 mintes at 5^{o}C to obtainedplasma. The clear supernatant (plasma) obtained was kept frozen at -15^{o}C for the estimation of plasma metabolites and enzymes.

Preparation of cytosolic fraction: The rats were killed by cervical dislocation, the liver was immediately removed, blotted of blood stain, rinsed in ice cold 1.15 % of KCl , weighed, and homogenized in 4 volume of ice-cold 0.1 M phosphate buffer, (P^{H} 7.4). The homogenates were centrifuged at 3000 rmp for 10 minutes at 4 ^{o}C using refrigerated centrifuge and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquot and used for enzyme assays. The supernatant was stored temperature of -15 ^{o}C for estimated of oxidative stress parameters.

Evaluation of hepatic and Renal function: These parameters were evaluated for liver function, alkaline phosphatase (ALP), alanine and aspartate aminotransferases (ALT and AST) following the method of (Reitman and Fankel, 1957; Belfield and Goldberg, 1971) respectively.According to Walter and Gerade (1970), the concentrations of bilirubin were tested in a university laboratory. Urea, creatinine, and lipid peroxidation were measured using UV/visible spectrophotometers in accordance with standard procedures according to Fawcett and Scott (1960) and Schirmeister *et al.*, (1964), respectively, and (MDA) formation was examined in accordance with standard procedures according to Ruiz-Larrea *et al.,* (1994).

RESULTS AND DISCUSSION

Tamoxifen involves its interaction with oestrogen receptor. This interaction is responsible for the pharmacological effects of tamoxifen (Roy*et al.*, 2014).Based on this study's findings (Table 1 and Fig. 1), tamoxifen pointedly harms the kidneys and liver in addition, changing the liver's antioxidant status. Co-treatment with trans-ferulic acid, however, acts as a defense agent against these harms. The concentrations of urea, creatinine, and bilirubin in the plasma were showed elevated in the group treated with tamoxifen, which suggests an impairment of renal and liver functions. The result showed that the effect of Trans-ferulic acid on Tamoxifen induced stress in the concentrations of plasma *urea*, creatinine and bilirubin in rat. Tamoxifen pointedly increased the concentrations of plasma urea, creatinine and bilirubin by 130.2**%,** 46.3% and 60.1% correspondingly, with relative to the control ($P<0.05$). However, the treatment of trans-ferulic acid significantly decreased the elevated plasma bilirubin, urea and creatinine

concentrations in relative to tamoxifen treated group (P<0.05). But trans-ferullic acid administration with tamoxifen was able to ameliorate this liver impairment.

Table. 1: Effects of Trans-Ferulic acid on Tamoxifen Induced Stress in the Levels of Plasma Urea, Creatinine and Bilirubin in Male Albino Rats (mg/dl)

	Urea (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl)
Control	14.7±0.31	0.98±0.05	3.31±0.15
TMX	35.5±1.8	2.15±0.06	7.80±9.29
TFA	21.2±0.36	1.07±0.03	3.65±0.18
TMX+TFA	26.2±0.45	1.26±0.02	4.42±0.1

TMX= Tamoxifen; TFA= Trans-ferulic acid. Values are Means ± SD for six rats in each group,significantly different from the control (P<0.05).

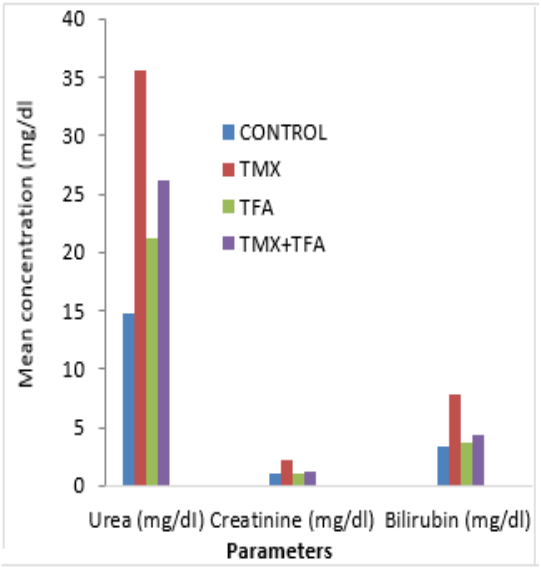

Fig 1. Effects of trans-ferulic acid on tamoxifeninduced variations in the concentrations of Plasma Urea, Creatinine and Bilirubin in Rats.
Where Tamoxifen (TMX) = (0.4 mg/kg body weight), (TFA)Transferulic acid =(100 mg/kg body weight) and (TMX+TFA)=(0.4 mg/kg + 100 mg/kg). N=6

The increased of the plasma levels of creatinine and urea is an indication of abnormal renal function comparing, (Shahidi and Zhong, 2010) Investigatedeffects of tamoxifen in rats in China, it was observed, that tamoxifen increased the plasma marker enzymes. The level of plasma AST, ALT and ALP activities reflect damage to hepatocytes and indicated the increased cellular permeability (El-Beshbishy*et al.*, 2010). However, in this study, the effects of trans-ferullic on tamoxifen treated rats was observed, tamoxifen increased the activities of plasma marker enzymes, as expected. But the effect of trans-ferulic acid on tamoxifen-induced varied in the activities of plasma AST, ALT and ALP in rats is presented in (Table.2 and figure 2). Tamoxifen pointedly increased themean concentrations of ALT, AST and ALP by 65±1.0 U/L, 11.08±0.12 u/l, and 678.20±13.3 u/l respectively in comparative with the control mean concentrations ALT, AST and ALP of 32.8±1.1 UL, 4.79±0.24 U/L and 415.7±5.4 U/L, while co-treatment of trans-ferulic acid with tamoxifen significantly ameliorated the elevated plasma AST, ALT, and ALP concentrations of 51.8±1.8 u/l, 7.5±0.32 u/l and 561.769 u/l.

Table 2. Effects of trans-ferulic acid on tamoxifen induced variations in the activities of plasma Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and AlkalinePhosphatase (ALP),

	AST (U/L)	ALT (U/L)	ALP (U/L)
CONTROL	32.8±1.1	4.79±0.24	415.7±5.40
TMX	65.0±1.0	11.08±0.12	678.20±13.30
TFA	43.6±1.0	5.54±0,26	461.8±3.20
TMX+TFA	51.8±1,8	7.5±0.32	561.769

Values were reported in mean and standard deviation of mean (M± SD.Tamoxifen (TMX) = (0.4 mg/kg body weight), the mean and standard deviation for six rats in each group, significantly different from control (P<O.05)

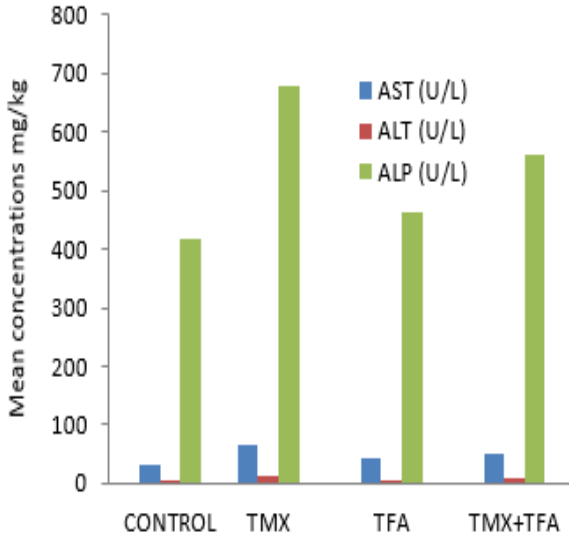

Fig:2: Effects of trans-ferulic acid on tamoxifen induced variations in the activities of plasma Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP),Where Tamoxifen (TMX)= (0.4 mg/kg body weight), (TFA)Transferulic acid =(100 mg/kg body weight) and (TMX+TFA)=(0.4 mg/kg + 100 mg/kg). n=6

The highest mean concentrations was observed the of ALP in the group treated with tamoxifen with mean concentration of 678.20±13.3 u/l, this study is similar to Hagar et al., (2020) reported a significant increase in the concentration levels of alkaline phosphatase,aspartate aminotransferase, and alanine aminotransferase activities and bilirubin, urea, and creatinine by administration (45 mg/kg bw) of tamoxifen to female wistar rats which was significantly reduced by 0.2 mg/kg bw of Sage oil which acted as an antioxidant.

Table 3: Effects of trans-ferulic acid on tamoxifen induced variations in the activities of Superoxide Dismutase (SOD) and Catalase in rats**.**

Treatment	Superoxide Dismutase (Units)	Catalase (µmole H_2O_2 consumed/min)
CONTROL	49.0±1.15	1.37±0.02
TMX	32.5±2.30	0.50±0.05
TFA	44.0±1.40	1.26±0.05
TMX+TFA	40.7 ± 1.60	1.08 ± 0.01

Values were reported in mean and standard deviation of mean (M± SD.Tamoxifen (TMX) = (0.4 mg/kg body weight), The mean and standard deviation for six rats in each group, Significantly different from control (P<O.05)

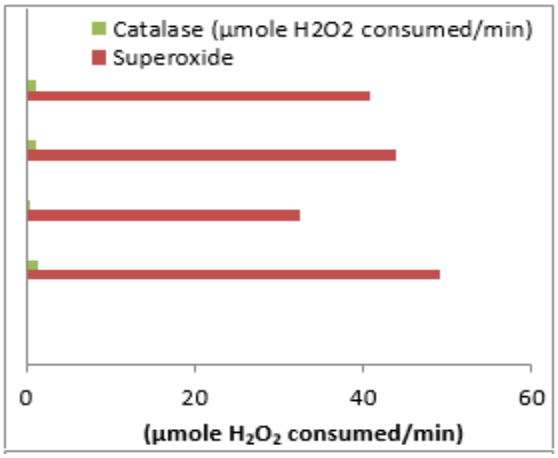

Fig: 3: Effects of trans-ferulic acid on tamoxifen induced variations in the activities of Superoxide Dismutase (SOD) and Catalase in ratsWhere Tamoxifen (TMX)= (0.4 mg/kg body weight), (TFA)Transferulic acid =(100 mg/kg body weight) and (TMX+TFA)=(0.4 mg/kg + 100 mg/kg).

The mean concentrations of the effects of trans-ferulic acid and tamoxifen-induced changes on hepatic superoxide dismutase (SOD) and Catalaseactivites in rats are presented in (table 3). Hepatic SOD activity was significantly reduced in tamoxifen treated group by mean concentrations of 32.5±2.30 µmole and 0.50±0.05µmolewhen compared with control (P<0.05),49.0±1.15µmole and 1.37±0.02µmole.Similarly, the concentrations of co-treatment with trans-ferulic acid significantly ameliorated the decrease in hepatic (liver) SOD and Catalase when compared with the tamoxifen treated group (P<0.05).There was a significant reduction in liver activities of superoxide dismutase (SOD), catalase (CAT), as a result of tamoxifen treatment in this study. The antioxidant enzyme SOD, catalase represent the guard mechanism against oxidative stress (Li *et al.*, 2012). SOD catalyse posed a rapid dismutation of superoxide radical in the body to hydrogen peroxide and oxygen gas while the hydrogen peroxide formed in this process and other cellular processes are converted by CAT into water and molecular oxygen. It is generally accepted, that H_20_2 can be detoxified by catalase which removes it when present at high concentration. Therefore, the reduction in the activities of SOD, Catalase by tamoxifen may predispose the liver to oxidative damage (Mürdter,*et a1* 2011).

Table 4: Effects of trans-ferulic acid on tamoxifen-induced variations on liver lipid peroxidation (MDA) in Rats.

	Hepatic lipid peroxidation(MDA) (Units)
CONTROL	16±0.38
TMX	97.9±6.90
TFA	22±0.70
TMX+TFA	81.4 ± 3.20

Values were reported in mean and standard deviation of mean (M± SD.Tamoxifen (TMX)= (0.4 mg/kg body weight),The mean and standard deviation for six rats in each group, Significantly different from control (P<0.05)

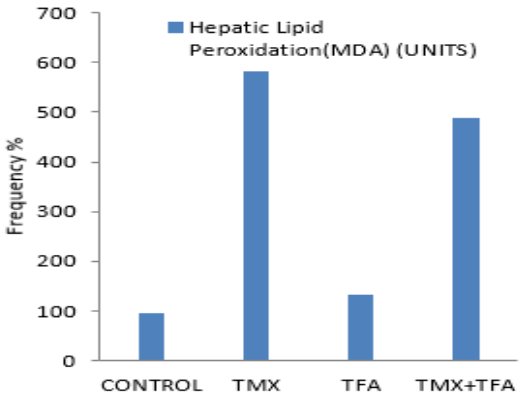

Fig 4: Effects of trans-ferulic acid on tamoxifen-induced changes on hepatic lipid peroxidation (MDA) in Rats.
Where Tamoxifen (TMX) = (0.4 mg/kg body weight), (TFA)Transferulic acid =(100 mg/kg body weight) and (TMX+TFA)=(0.4 mg/kg + 100 mg/kg) (TFA)Transferulic acid =(100 mg/kg body weight) and (TMX+TFA)=(0.4 mg/kg + 100 mg/kg)

Table 5. Effects of trans-ferulic acid on tamoxifen induced variations on the concentration of hepatic ascorbic acid in rats**.**

Treatment	Hepatic ascorbic acid (UNITS)
CONTROL	60.48±1.45
TMX	41.7±2.90
TFA	55.44±1.76
TMX+TFA	51.28 ± 2.02

Values were reported in mean and standard deviation of mean (M± SD. n=6.Tamoxifen (TMX) = (0.4 mg/kg body weight), the mean and standard deviation for six rats in each group, significantly different from control (P<O.05)

Reports have shown that transferulic acid protects the activities of both enzymes anddetoxifies H_20_2 into water. Ascorbic acid is key player in the line of antioxidant defense in the body ((Li *et al.*, 2012), and this vitamin is likely to be most susceptible to free radical oxidation. They are good free radical scavenger due their chemical properties. Studies have shown that the redox state of intracellular vitamin C is controlled by the intracellular level of OSH (Mortezavi, *et al.*, (2020).The decrease in the antioxidant status of the rat imply an increased susceptibility of the liver issues to radical species generated by the drug.

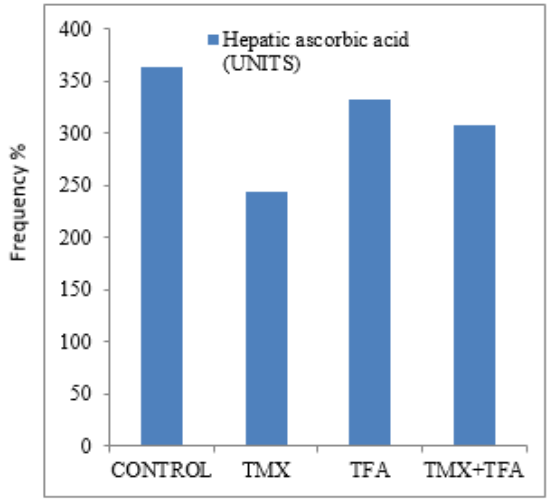

Fig 5: Effects of trans-ferulic acid on tamoxifen induced variations on the concentration of hepatic ascorbic acid in rats**.**
Values were reported in mean and standard deviation of mean (M± SD. Tamoxifen (TMX)= (0.4 mg/kg body weight), The mean and standard deviation for six rats in each group. Where Tamoxifen (TMX)= (0.4 mg/kg body weight), (TFA)Transferulic acid =(100 mg/kg body weight) and (TMX+TFA)=(0.4 mg/kg + 100 mg/kg).

The effect of Trans-ferulic acid on Tamoxifen-induced changes on the concentration of hepatic ascorbic acid following treatment with tamoxifen (Table 5 and Fig 5). The ascorbic acid mean concentrations was significantly decreased, in tamoxifen treated group by 41.7±2.9 U/L when compared with the mean concentration of the control (60.48±1.45 U/L). The combined treatment of tamoxifen and trans-ferulic acid significantly attenuated the decrease in hepatic Vit. C when compared with the tamoxifen treated group ($P<0.05$) with mean concentration of (51.28 ± 2.02 U/l). Membrane lipids succumb easily to deleterious actions of reactive oxygen species (Shih,Yeh,andYen,2010).The measurement of lipid peroxidation is a convenient method to monitor oxidative damage (Mortezavi, *et al.,* 2020). In the present study, the increased concentration of thiobarbituric acid reactive substance TBARS (malondialdehyde) in the liver of rats treated with tamoxifen showed a consequence of oxidative stress by lipid peroxidationcaused by the drug. Treatment with trans-ferulic acid protected the liver cell through reduction of lipid peroxidation and decreased the production of free radical derivatives as evident from the decreased concentration of liver TBARS, Thus trans-ferulic acid offered protection against oxidative stress by scavenging of free radicals. The activities of the antioxidant also reported to protect the liver against LPO and membrane disintegration during hepatocarcinogenesis in rats (Chang *et al.,* 2013)
Conclusion: Tamoxifen induced marked renal and hepatic damages, depleted the antioxidant status of the liver resulting in oxidative stress. However, trans-ferullic acid, a potent antioxidant that protects against the oxidative stress induced by tamoxifen. Based on this findings and the conclusions from this study the use of tamoxifen for treatment of health issues should be controlled or monitor by the appropriate agencies or encourage the used with an antioxidant, it is therefore, strongly recommended that more research should be encouraged for the discovering of more natural antioxidant that can overturn the effects of oxidative stress induced by drugs.

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