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Original Article

In vivo EVALUATION OF WEAVER ANT *(Oecophylla smaragdina)* EXTRACTS ON HEMATOLOGICAL STATUS AND LIVER FUNCTION INDICES OF *Plasmodium berghei* INFECTED MICE

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ABSTRACT

Malaria parasite multiplication and the influence of drug dosage have been well addressed as chief bases for some pathological symptoms and proper functioning of essential body organs, respectively. This study therefore elucidated the effect of methanol extract of Weaver ant (*Oecophylla smaragdina*) on some serum and liver enzymes, as well as, haematological variables in *Plasmodium berghei*-infected mice. Fifteen mice were randomly allocated into six groups of three animals each. Group I and II were given normal saline (2 ml/kg) and chloroquine (5 mg/kg) to serve as untreated and treated controls, respectively. Groups III-V received the extract at 150, 300 and 600 mg/kg body weight, respectively. All treatments were given through the oral route. Haematological and biochemical parameters including alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities and total protein concentration in serum and liver of mice were evaluated following standard procedure. Results indicated that infected untreated mice had significant ($p< 0.05$) decrease RBC, Hb, PCV, MCV and TWBC level when compared with the normal control and mice treated with 600 mg/kg of extract. Infected untreated mice also show significant ($P<0.05$) decrease in the activities of AST and ALT in liver and increase in the serum. The ALP activities and total protein increased significantly ($P<0.05$) in liver relative to the normal control. However, treatment of the infected mice with graded dose of the *O. smaragdina* caused a reversal in the pattern of these parameters in a dose-dependent manner comparable to that of the standard drug. This study indicates that n-Hexane extract of *O. smaragdina* contain principles that may be useful in the management of anaemia and liver disorders elicited by *Plasmodium berghei* infection.

Keywords: *Plasmodium berghei, Oecophylla smaragdina,* haematological,

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INTRODUCTION

Malaria infection remains the major public health threat of developing countries, despite intensive integrated effort to curb the disease, in the past few decades. Uncontrolled resistant development of the malaria parasite to available treatments/drugs and the vector mosquitoes to insecticides has caused set back to decrease in the malaria morbidity and mortality (Koffi *et al.,* 2011; Konstantinos *et al.,* 2018). Pregnant women and children under the age of 5 years has been identified as the main victim of malarial burden (CDC, 2018).

Malaria infection, the major infectious diseases that painstakingly affect people, especially in the tropical and sub-tropical regions of the world (Caraballo, 2014) is a mosquito-borne disease caused by the intraerythrocytic protozoa parasite of the genus *Plasmodium (*i.e., *P. falciparum, P. vivax, P. ovale,* and *P. malariae* and *P. knowelsi*) (Johnson *et al*., 2017). Although, the multiplication of the *Plasmodium* parasite within the red blood cells of the host causes pathology such as anaemia and damage of essential organs including, lungs, liver and spleen (Ibiam *et al*., 2017), the dysfunction of some organs in the body could also have been linked to the antimalarial drugs used during the treatment of malaria infection. Adetutu *et al*. (2015) reported that the antimalaria drug dosage used could have effect on the liver and kidney functioning. It is therefore very pertinent to ascertain the effect of any ingestible extract on the serum activities of the liver enzymes so as to ensure the hepato-protectiveness of such extract or drug, during treatment of malaria infection (Akanbi *et al*., 2017). Liver function tests, which include estimation of plasma protein, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin and total protein (Shittu *et al*., 2013) have been well exploited in the hepatoprotectiveness of natural product extracts (Johnson *et al.* 2017; Shittu *et al.,* 2017).

Africa is considered to be the cradle of mankind which is rich in biological and cultural diversity of natural products for healing practice (Bashir *et al*., 2015). Insect and insect products have been cited for antimalarial, antitrypanosomal activities, as well as, their protective role against organs damage during parasitic infection (Shittu *et al*., 2013; Shittu and Eyihuri, 2015; Lawal *et al*., 2015; Shittu *et al.,* 2018). *Oecophylla smaragdina* (weaver ant) is known for an aggressive painful bite and as a predator of various insect species (Ganesh and Deepak, 2016).The traditional healers in Thailand, India and African use these ants to treat common health problems and as an ingredient in some food items (Oudhia, 2002; De Foliart, 2008). *Oecophylla smaragdina w*hole-body aqueous extract of worker ants has been shown to possess significant antioxidant and anti-arthritic properties.. GC-MS analysis of abdominal glands revealed the presence of 39 chemical compounds with antiparasitic reputations (Vidhu and Evans, 2015). However, literature survey revealed no any documentation on it protective effect during malaria complications. With the advocacy of WHO for traditional based therapy, this work therefore evaluated the effect of methanol extract of *Oecophylla smaragdina* on biochemical and hematological variables in mice experimentally infected with *P. berghei.*

MATERIALS AND METHODS

Collection and Processing of *Oecophylla smaragdina*

Large nests of *O. smaragdina* were collected from the field at Gidan Mangoro, Bosso Local Govt., Minna, Niger State. The

insect were put in a glass jar and then transferred to the Laboratory of Department of Biological Science, Federal University of Technology Minna, Niger State, where it was identified and authenticated by a senior entomologist. The insect were killed in freezer at -4^{0}C and water droplet and/or ice debris were removed by desiccation under activated desiccant. Complete drying was done at room temperature (Adebayo *et al*., 2003).

Extraction

The air-dried samples (100 g) were extracted with 500 mL of absolute methanol via cold extraction. The extract was filtered through a Buchner? funnel using Whatman no. 1 filter paper. The solvent was evaporated under reduced pressure in air at 30^{0}C (Shittu *et al.,* 2013).

Experimental Animals

Healthy albino mice of average weight 22-25g were purchased from small animal holding unit of School of Life Sciences, Federal University of Technology, Minna, Niger State Nigeria. The rodents were housed in standard environmental conditions of 70% relative humidity, (27 $\pm$ 2.0) °C of temperature, 12 h night/day light cycles, free water access and pellets. All experimental procedures involving animals were conducted in accordance with Canadian Council on Animal Care Guidelines and Protocol Review and approved by the Federal University of Technology, Minna Committee on ethics for medical and scientific Research..

Reagent and Assay kits

The assay kits for T. protein, AST, ALT and ALP were products of Randox Laboratories Ltd, United Kingdom. All other reagents used were of analytical grade and were prepared in distilled water.

Parasites

Plasmodium berghei NK65 chloroquine sensitive strain was obtained from NIPRD Abuja, Nigeria and maintained in our laboratory by serial passage in mice.

Acute Toxicological Studies

Acute toxicological study was carried out to determine the median lethal dose (LD_{50}) in accordance with Lorke's method (Lork, 1983).

In vivo antiplasmodial study*:*

Standard inoculums of 1 x 10^7 *P. berghei* infected red blood cells were injected into mice intraperitoneally. Seventy-two hours later, the mice were divided into five groups of three mice each. The negative control received distilled water and the positive control received chloroquine (5 mg/kg) and the test groups received 150 mg/kg, 300 mg/kg and 600 mg/ kg body weight of *O. smaragdina*. All treatment was administered orally for 5 days.

Determination of Haematological Parameters

The haematological components including haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), Granulocyte count (GRA), lymphocytes (LY), plate late count (PLT) *and* differential count were determined using the automated hematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (1995).

Serum and Liver Collection

Mice were anaesthetized in slight chloroform through cardiac puncture. The blood were collected into a centrifuge tube and allowed to stand for 10 minutes and then centrifuged at 1000 rpm for 15minutes. The supernatant (serum) was carefully removed and stored until needed for further analysis. The liver were excised and transferred into ice-cold 0.25 M sucrose solution. This was later homogenized in ice-cold 0.25 M sucrose solution [1:5w/v] and the supernatant were used for further studied.

Serum and Liver Biochemical Assay

The enzymes: alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were assayed using standard procedures (Reitman and Frankel, 1957). Total protein concentration was determined using Biuret method described by Gornall *et al.* (1949). All measurements were done using UV Spectrophotometer.

Data Analysis

Results were expressed as multiple comparison of Mean $\pm$ SEM. Significance difference was determined using One-way Analysis of Variance (ANOVA) coupled with Duncan Multiple Range test. A probability level of less than 5% was considered significant (Adamu and Johnson, 1997).

RESULTS

Acute oral toxicity

The Acute oral toxicity profile of the extract is presented in Table 1. With the acute toxicity test at the limit test dose of 5,000 mg/kg, neither mortality nor significant changes related to behavioural, autonomic and neurological profile were observed within the first 24 h. The physical signs of toxicity observed at doses of 2900 and 5000 mg/kg body weight included excitation, paw licking and increased respiratory, these however, disappears within the 24 h of extract administration. The LD_{50} can therefore be extrapolated to be greater than 5000 mg/kg bity weigh.

Table 1: Acute Oral Toxicity profile of methanol extract of *O. smaragdina* in mice

Group	**Extract dosage (mg/kg b. wt)**	**Mortality/no. of mice per group**	**Sign of toxicity**
1	10	0/3	None
2	100	0/3	None
3	1000	0/3	None
4	1600	0/3	None
5	2900	0/3	Excitation, paw licking and increased respiratory, these however, disappears within the 24 h
6	5000	0/3	Excitation, paw licking and increased respiratory, these however, disappears within the 24 h

Effect of Methanol Extract of *O. smaragdina* on Haematological parameters in *Plasmodium berghei*-infected Mice

The effect of methanol extract of *O. smaragdina* on hematological parameters in *P. bergei* infected mice is detailed in Table 2. Infected untreated mice showed significant ($p< 0.05$) decrease in RBC, Hb, PCV, MCV and TWBC compared with the normal control and mice treated with 600 mg/kg of extract. There were no significant difference ($P<0.05$) in RBC, Hb, PCV, MCV and TWBC recorded for the group treated with 150, 300 mg/kg extract and the untreated group. No significant difference ($p>0.05$) in MCH and MCHC was observed among all the experimental groups.

Table 2: Effect of methanol extract of *O. smaragdina* on hematological parameters in *P. bergei* infected mice

Treatment (mg/kg b. wt)	HB	PCV (%)	MCV (FL)	MCH (pg)	MCHC (gL)	RBC ($x10^{22}$/L)	PLC ($x10^{9}$/L)	TWBC ($x10^{9}$/L)
150	8.25 ± 0.05^{a}	26.90±0.54a	39.01 ± 0.01^{a}	14.01 ± 0.01^{a}	35.50 ± 0.50^{a}	4.20 ± 0.10^{a}	980.00 ± 9.00^{b}	3.01±0.01a
300	8.06 ± 0.00^{a}	26.90 ± 0.21^{a}	38.92 ± 0.56^{a}	14.00 ± 0.00^{a}	35.00 ± 1.00^{a}	5.25 ± 0.05^{a}	934.50 ± 8.50^{b}	3.55 ± 0.05^{a}
600	14.00 ± 0.10^{c}	38.95 ± 0.44^{b}	44.00 ± 0.00^{b}	15.00 ± 0.32^{a}	34.01 ± 0.01^{a}	5.80 ± 0.20^{a}	925.50 ± 9.50^{b}	6.20 ± 0.20^{b}
Standard	13.81 ± 0.01^{b}	39.28 ± 0.56^{b}	44.00 ± 1.00^{b}	15.02 ± 0.01^{a}	35.00 ± 0.00^{a}	7.71 ± 0.01^{b}	479.00 ± 4.00^{a}	8.10 ± 0.20^{e}
Untreated	9.00 ± 0.00^{c}	29.76 ± 0.52^{a}	34.45 ± 0.21^{a}	15.16 ± 0.21^{a}	35.50 ± 0.40^{a}	5.35 ± 0.20^{a}	541.04±3.67a	3.27 ± 0.29^{a}
Normal	14.90 ± 0.12^{d}	39.76 ± 0.87^{b}	43.00 ± 0.00^{b}	16.01 ± 0.01^{a}	32.00 ± 0.50^{a}	9.35 ± 0.07^{c}	558.00 ± 2.00^{a}	7.10 ± 0.10^{d}

Values are expressed as means = Standard error of three replicates

Values followed with the same superscript alphabets on the same column are not significantly different at $p>0.05$

Keys: HB= Haemamoglobin, MCHC= Mean Corpuscular hemoglobin Concentration, RBC= Red blood cell count; WBC= White blood cell count; PLC= Platelet count.

Effect of Methanol Extract of *O. smaragdina* on Liver Function indices in *Plasmodium berghei*-infected Mice

Infection of the mice with *P. berghei* caused significant ($P<0.05$) decrease in the activities of AST (Figure 1) and ALT (Figure 2) in liver and increase in the serum. ALP (Figure 3) activitiesand total protein (figure 4) increased significantly ($P<0.05$) in liver relative to the normal control. However, treatment of the infected mice with graded dose of the *O. smaragdina* (150, 300 and 600 mg/Kg) caused a reversal in the pattern of this parameters in a dose-dependent manner during the treatment period to a level comparable to the level observed among the standard control group. The effect of the extract, especially the highest dose of 600 mg/Kg body weight was comparable to that of the standard drug as there was no significant difference ($P>0.05$) in most of the parameters between the groups

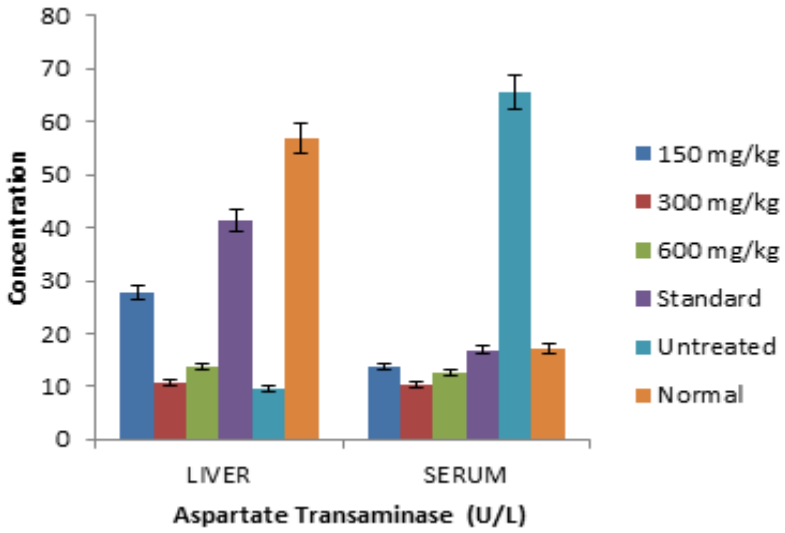

Figure 1: Serum and Liver AST Activity in Mice Treated with methanol Extract of *O. smaragdina*

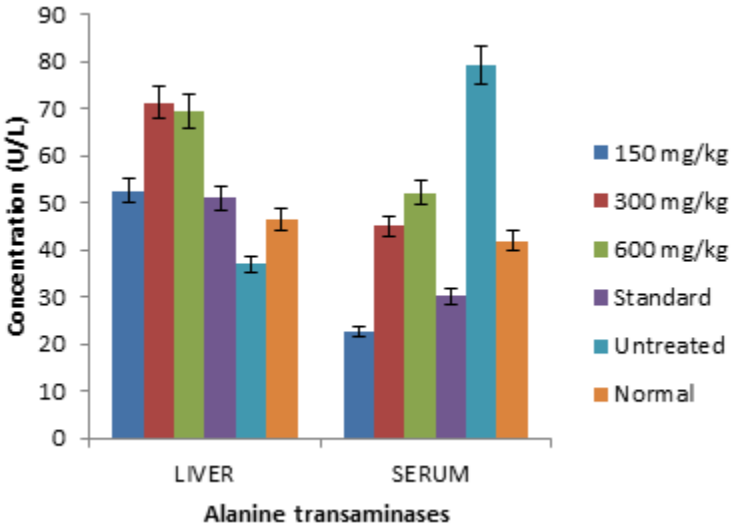

Figure 2: Serum and Liver ALT Activity in Mice Treated with methanol Extract of *O. smaragdina*

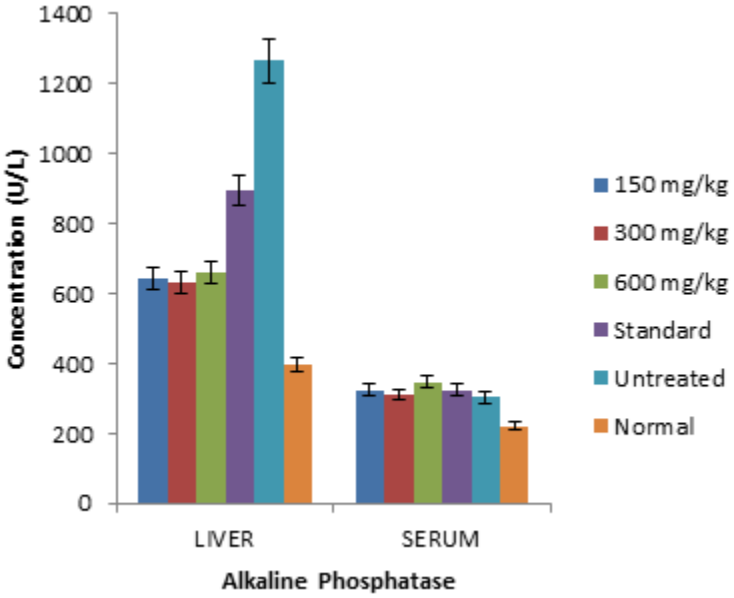

Figure 3: Serum and Liver ALP Activity in Mice Treated with methanol Extract of *O. smaragdina*

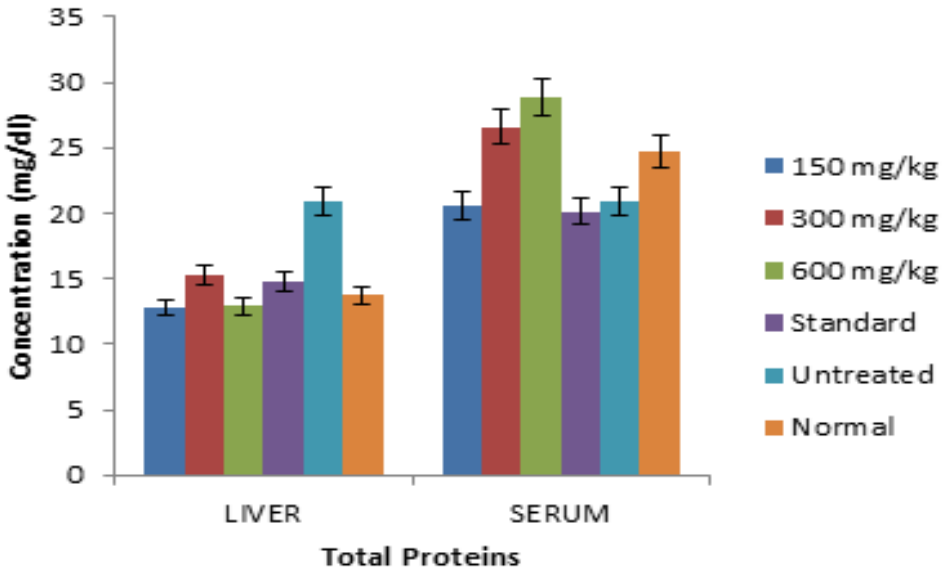

Figure 4: Serum and Liver total proteins in Mice Treated with methanol extract of *O. smaragdina*

DISCUSSION

Acute toxicity tests are generally the first tests conducted in any toxicity study. They provide data on the relative toxicity likely to arise from a single or brief exposure to any substance. Different insect/venom/extracts have been known to possess different levels of toxicity which majorly depends on the levels of poisonous agents inherent in them (Sofowora, 1993). Safety profile assay of the methanol extract of *O. smaragdina* in mice revealed an oral median lethal dose (LD_{50}) extrapolated to be greater than 5000 mg/kg body weight which is the maximum allowable dose by the Organization for Economic Cooperation and Development (OECD) guideline for testing of chemicals (OECD, 2008). This result suggests that the methanol extract of *O. smaragdina* is relatively non-toxic since LD_{50} above 5000 mg/kg body weight is of no practical significance (Lorke, 1983). This is expected considering that the spice is edible. The non-lethal effects produced with the high dose of this extract are an indication that the extract of *O. smaragdina* is relatively safe on acute oral exposure. It can therefore be concluded that *O. smaragdina* extract is non-toxic which is in agreement with Bruce (1987), Salim *et al.,* (2016) and ASTME, (1987), that any chemical substance with LD50 estimate greater than 3000-5000 mg kg-1 (oral route) could be considered of low toxicity and safe, this is also in agreement with OECD (2008).

Generally, analyses of the activities of some basic liver function enzymes in the plasma or serum can be used to indirectly access the integrity of tissues during malarial infection or after being exposed to certain pharmacological agent (Momoh *et al.*, 2014). These enzymes are usually biomarkers whose plasma concentrations above the homeostatic limits could be associated with various forms of disorders which affect the functional integrity of the liver tissues (Lawal *et al.*,

2016). Infection of the mice with *Plasmodium berghei* also caused significant ($P<0.05$) increase in ALT and AST activities relative to the normal control. This result is in consistent with studies which reported that majority of malaria patients showed elevated serum activities of AST, ALT and ALP which is an indication of liver damage (Uraku, 2016). However, treatment of *P. berghei*-infected mice with the methanol extract of *O. smaragdina* at the tested dose showed a significant ($P<0.05$) reduction in activities of these parameters. This finding correlates with previous findings. Olorunnisola and Afolayau (2013), reported that treatment of parasitized mice with leaf extract of *S. jollyanum* had a significant ($P<0.05$) reductions in elevated levels of AST, ALT, ALP, and GOT when compared with parasitized non-treated group (PNT). According to Babamale *et al.* (2017), decreased activities in liver enzyme such as AST and ALT in the investigated untreated infected mice are strong indication of pathological condition of malaria infection due to the pre-erythrocytic activities of the sporozoite. This may, in addition, ascribed to significant perturbation of organ's parenchyma and membrane which leads to leakage of organ's enzyme into the blood circulation. (Koichi *et al*, 2013).

Heamatological changes are some of the most common complications in malaria and they play a major role in malaria pathology (Maina *et al.,* 2010). The major function of red cells is to contain haemoglobin, which in turn carries oxygen from the lungs to the tissue and carbondioxide from the tissues to the lungs (Waugh and Granti, 2001). In this study there was a significant reduction in RBC, Hb, PCV, MCV and TWBC of untreated *P. berghei* parsitized mice which could have predisposed the animals to anaemic condition (Muhammed and Oloyede, 2009). Significant increase in RBC, Hb, PCV, MCV and TWBC of the extract (600 mg/kg) treated parsitized mice in this study implied that the extract demonstrated an anti aneamic property. White blood cells count are generally observed to be lower during malaria infection (White and Breman, 2001). Decreased in WBC count in the untreated *P. berghei* parsitized mice in this study was an indication of malaria infection. Whereas there were increase in WBC count in extract-treated mice close to normal indicating that the extract demonstrated antiinflamatory property (Abubakar *et al*., 2016). However, treatment at doses of 150 and 300 mg/kg of the extract fail to prevent *P. bergei* induce anemic condition.

According to Manzur (2009), higher concentration of extract from natural products showed appreciable inhibitory effect against *P. bergei* induced complications. This was in line with the results of the present study as Increase in the concentration of the extract yielded their corresponding increase in antianaemic effect. This linear relationship between the concentrations of extract and antimalarial activities reflect increase in the amount of active principle as the concentration of the extract increase, so more of the active agents were able to diffuse into the cell.

Conclusion

The extracts medial lethal concentration establishes the oral safety of the extract. This investigation indicates the tendency to restore liver and kidney impairments, as well as, ability to moderately ameliorate the inhibitory effect of malaria

parasite on the synthesis of liver enzymes. The extract also possesses antiplasmodial potency to reduce parasitaemia induced alteration in haematological parameters. Further studies to identify the bioactive metabolites of the extracts, as well as, investigate their mechanism of action are recommended.

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