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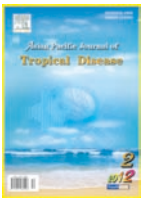

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Preliminary studies on the antiplasmodial potential of aqueous and methanol extracts of *eucalyptus camadulensis* leaf

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ABSTRACT

Objective: The rising problem of resistance to the classical drugs like chloroquine and the problem of recrudescence of malaria after treatment with artemisinin suggest the need for new antimalaria agents. This project was designed to explore the antiplasmodial potential of *Eucalyptus camaldulensis* leaf extracts. **Methods:** The antiplasmodial potential of the methanol and aqueous extracts of Eucalyptus camadulensis (leaf) were evaluated in a mouse model of malaria. Swiss albino mice were intraperitoneally infected with *plasmodium berghei* (NK65), a rodent malaria parasite. The level of parasitemia, life span, variation in weight and percentage packed cell volume (% PCV) of infected and treated mice were used to assess the efficacy of extracts. Treatment with the extracts at dose levels of 100, 200 and 400mg/kg body weight commenced 72 hours post infection for the test groups, while a standard antimalarial drug, Artesunate, at a dose of 50mg/kg body weight was administered on the positive control group. The negative control group was left untreated. **Results:** Animals treated with the methanol extract showed a significant decrease in parasitaemia ($p < 0.05$), and survived for 29 days compared with those treated with the aqueous extract which survived for 19 days with a higher level of parasitemia. However, the control group treated with Artesunate showed a significantly lower parasitaemia ($p < 0.05$) and survived for 34 days when compared with the groups treated with methanol and aqueous extracts. The level of parasitemia, decrease in weight and %PCV in all the treated groups was significantly lower ($P < 0.05$) compared with the infected but untreated group (negative control) which survived for only 7 days. **Conclusions:** The methanol extract of the leaves of E. camadulensis has an antimalarial potential that could be exploited for the benefit of mankind.

1. Introduction

Malaria remains a serious global health burden, with an annual incidence of 247 million cases and nearly one million deaths, most of which afflict children living in Africa [1]. It is caused by blood-borne parasite of the genus plasmodium which is transmitted to humans through the bite of female anopheles mosquito [2]. One of the major ailments that are of concern in the world today is malaria. Malaria is the single most important cause of ill health, death and poverty in most African countries [3]. The disease is believed to be a major obstruction to social and economic development in Africa, causing enormous misery and suffering through the pain of fever and anguish of bereavement. It remains one of the major killers of humans worldwide, threatening the lives of more than one third of the world's population. It thrives in the tropical areas of Asia, Africa, and Central and South America, where it strikes millions of people. Malaria is prevalent in these regions because of the significant amounts of rainfall and consistent high temperatures; warmth, consistent temperatures and high humidity, along with stagnant waters in which plasmodium larvae mature, which provide mosquitoes with the environment needed for continuous breeding.

Each year, 300 to 500 million cases of malaria occur worldwide and 1.2 – 2.8 million deaths due to malaria occur annually [4]. Sadly, young children with naïve immune systems and pregnant women with potentially compromised immune systems are particularly vulnerable to this disease and so are considered to be the highest risk populations for malaria-related deaths. *P. falciparum* disease severity ranges from severe and complicated, to mild and uncomplicated, to asymptomatic [5]. Understanding the impact of *P. falciparum* on the human host across this range

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is critical for learning how to improve the management of the disease. More than 1 million of its victims, mostly young children, die yearly, accounting for ninety percent of death in children aged less than five years [1].

Urbanization is increasing in Africa and is resulting in a change in the epidemiology of malaria. Currently, 40 African cities have more than one million inhabitants and in 2003, 39% of Africans lived in cities. While urban malaria morbidity has been estimated to be between 25 and100 million cases, amounting to between six and 28% of the total annual incidence [6]. According to the United Nations (UN) projections, by 2025, over 800 million people, about 54% of the continent's population, will live in urban areas [6]. Thus, urban malaria has become an emerging public health problem in Africa and has become the subject of many studies to better understand its determinants, suggest preventive measures and appropriate control [7]. Thus, Urban malaria control programmes need to consider the socio economic level of an area rather than the location in the city in order to determine the areas most favourable to malaria transmission.

Malaria infection is characterized by defective immune responses. Severe malaria is a complex multisystem disorder, in which complications such as cerebral malaria, anaemia, jaundice, respiratory distress, and hyper parasitemia can occur [8]. The problem militating against the effective management of malaria are numerous. The first and most important problem is that plasmodia parasites are resistant to most widely available, affordable and safest, first line treatments such as Chloroquine and Fansider drugs [3]. Also the overall control of mosquitoes which transmit malaria is made difficult by their resistance to a wide range of insecticides. The third problem which is a new and rapid developing one is the wide production and distribution of fake antimalaria drugs. Fraudulent and substandard antimalarial drugs could be wrecking the chances of winning the war against malaria in Africa. Researchers from the Wellcome Trust-Mahosot Hospital-Oxford University Tropical Medicine Research Collaboration reported in the Malaria Journal that millions of lives could be lost over the next twelve months unless urgent action is taken both within the African continent and elsewhere in the world.

Fake medications are coming onto the scene as a result of deliberate criminal activity, while substandard drugs are becoming more common because of poor manufacturing practice. Not only are scores of patients being inadequately treated, but the presence of these undesirable and illegal medications significantly raises the risk of drug resistance among the malaria parasites. Approximately 781,000 people are thought to have died from malaria in 2009 [9].

Lastly, most countries in Africa lack the necessary infrastructure and resources to manage and control malaria [1]. There have been massive international efforts to distribute insecticide-treated bednets (ITNs) and the main focus in the promotion of their use has been on pregnant women and the under-fives. However, a study [10] in 18 African countries has found that the 5-19 year-old age group is the least likely to be protected by a net. One reason this is so worrying is that individuals in this age group who have been shielded by a net in their first few years may not have developed a sufficient level of immunity to protect them. Also, by harbouring large parasite numbers, they will in effect be acting as a major reservoir of infection, thus threatening other community members.

Before the advent of orthodox medicine, in all malaria endemic countries, plants are used for the treatment of malaria [11].

Eucalyptus camaldulensis (Myrtaceae), an Australian native, represented by around 700 species is a genus of tall, evergreen and magnificent trees cultivated the world over for its oil, gum, pulp, timber, medicine and aesthetic value. Among the various wood and non-wood products, essential oil found in its foliage is the most important one and finds extensive use in food, perfumery and pharmaceutical industry. In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal [12].

Crude methanol extract of Eucalyptus Camadulensis (Myrtaceae) has been reported to inhibit the growth of Candida albicans [13].

In some Northern Nigerian communities, the fresh leaves of *Eucalyptus camaldulensis*, known locally as 'Itchen Turare' is boiled and the decoction used in the treatment of malaria fever but this has not been scientifically verified. This study was designed to evaluate the antimalarial potential of the aqueous and methanol extracts of E. camadulensis (leaf) in infected mice models.

2. Materials and methods

2.1Materials

2.1.1 Plant collection and preparation

The leaves of Eucalyptus Camadulensis (NIPRD/H/6263) were collected from Niger state old secretariat in Minna, Niger state, Nigeria during the rainy season in the month of May. The leaves were washed, drained and air dried at room temperature for two weeks. The dried leaves were grounded into powered form using an electric blender as described by [14].

2.1.2 Animals

Swiss albino mice weighing 29 - 41g were obtained from Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. The mice were maintained on standard feed and water in compliance with the internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care, Guidelines on animal use protocol review [15].

2.1.3 Malaria parasite

Plasmodium berghei NK65 was obtained from the Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

2.1.4 Standard drug

The standard antimalarial drug, Artesunate, was obtained from Zagbayi Pharmacy, Minna, Nigeria.

2.2 Methods

2.2.1 Methanol and aqueous extraction

The powdered sample was extracted using the two solvents using the method described by [16]. The dry extract weighing 8.25g from methanol and 12.25g from water were transferred into sample bottles and refrigerated until required for use.

2.2.2 Infection of mice

Thirty two (32) Swiss albino mice were intraperitoneally administered with a standard inoculum of P. berghei diluted appropriately with normal saline to make 1×107 parasitized red blood cells, according to the method described by [3]. The level of parasitemia in the animals was ascertained after 3 days to allow for the attainment of a high level of parasitaemia. The parasitaemia count was carried out and the average percentage parasitaemia calculated using the method of Obih and Makinde as described by [3] in which: Average % Parasitaemia = No of parasitaemia / No of WBC × 100.

2.2.3 Administration of Extracts

Administration of extracts commenced three days post infection when symptoms like ruffled fur, sluggish movement or hunched sitting position; convulsions or ptosis (drooping of upper eyelids) and loss of appetite were observed in the infected animals and were based on dosage and extract type. The mode of administration was by oral cannular for 5 consecutive days. Eight groups, each consisting of four mice were set up. Groups 1 – 3 were administered 100, 200, and 300mg/kg bodyweight of aqueous extract of *E. camaldulensis* (leaf). Groups 4 – 6 were administered 100, 200, and 300mg/kg bodyweight of methanol extract of *E. camaldulensis* (leaf). Group 7 was administered 50mg/kg bodyweight of the standard drug, Artesunate while Group 8 was left untreated.

2.2.4 Monitoring the course of parasitemia

This was done according to the method described in (2.2.2) above. Blood was collected from the tails of infected mice and smears were made on glass slides. Using a microscope set at 40X, the numbers of parasites were estimated for all animals.

2.2.5 Monitoring changes in percentage packed cell volume (%PCV) and weight of test animals

The % PCV values and the weights of mice in all groups were noted before infection; 72 hours post infection and after 5 days of treatment.

2.2.6 Toxicity test

The acute toxicity of the aqueous and methanol extracts of *E. camaldulensis* leaf were tested using mice that were acclimatized and made to fast over night according to the method of [18]. Five mice per group were administered orally the two extracts separately at 1600, 2900 and 5000mg/kg bodyweight as a single dose. Signs of toxicity such as death, change in physical appearance and behaviour were recorded within 24 hours.

2.2.7 Phytochemical screening

The aqueous and methanol leaf extracts of E. camadulensis leaf were tested for the presence of terpenes, flavonoids, tannins, saponins and alkaloids using standard procedures as described by [18].

2.2.8 Statistical analysis

Data obtained in this study were analyzed using the statistical software SPSS v16.0 (SPSS Inc, Chicago, Illinois, USA). Numerical data were presented as mean ± standard deviation. The significance of the mean difference between two independent groups was determined using Student's t-test, and one-way analysis of variance (ANOVA), while multiple comparisons were used when comparing more than two groups. A p – value < 0.05 was considered significant.

3. Results

3.1 Anti-plasmodial activity of Extracts

3.1.1 Methanol Extract

Administration of the extract commenced 72 hours post infection at a time when parasitemia was established in all the animals. The courses of parasitemia in groups treated with doses of the methanol extract are presented on Table 1 and Figure 1. After 5 days of treatment, it shows that percentage parasitemia was significantly lower in the group treated with 400mg/kg bodyweight of the extract when compared with other dose groups thus indicating a dose – dependent relationship. However, % parasitaemia was significantly lower in the group treated with Artesunate (4.31±0.28) when compared with the group treated with the highest dose of the extract (14.00±1.08) ($P \leqslant 0.05$). On the 7th day, all animals in the negative control group were dead while no single death was recorded in the methanol extract – treated groups and the Artesunate – treated group

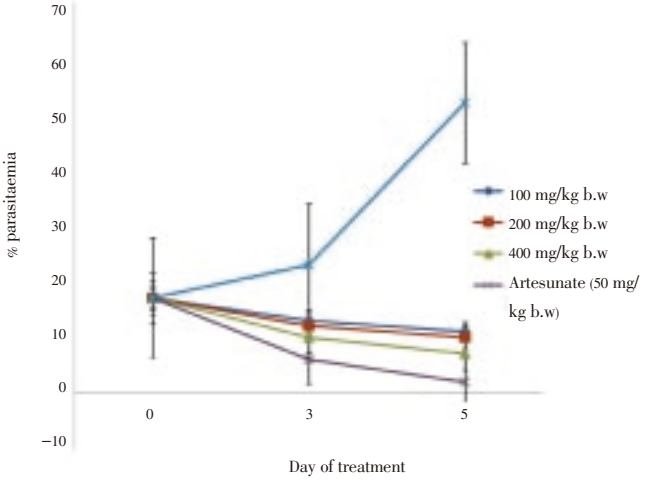

Figure 1. Course of parasitaemia in infected mice treated with methanol extract of *E. camaldulensis* and Artesunate.

3.1.2 Aqueous extract

The result of the antiplasmodial effect of the aqueous extract is shown on Table 2 and Figure 2, which indicate that after 5 days of treatment, the Artesunate – treated group had a significantly lower parasitaemia (4.31±0.28) than the group treated with the highest dose of the extract, 400mg/kg body weight ( ) ($P \leqslant 0.05$). The negative control group had a significantly higher level of parasites in circulation after five days (103.38 ± 4.88) compared to all the treated groups ($P \leqslant 0.05$). On the 7th day, all animals in the negative control group were dead while 50% death was recorded in the

Table.1

Level of Parasitemia in infected mice treated with methanol extract of *E. camaldulensis* (leaf)

Days of treatment	Dose	Mean Value	*P. value*	Average % parasitaemia
Day1	100mg/kg	34.06a±0.41	0.992	17.03
	200mg/kg	33.69ab±3.39	"	16.84
	400mg/kg	33.00abc±0.69	"	16.50
	Artesunate	34.13abcd±2.22	"	17.05
	Negative control	34.25abcde±0.85	"	17.12
Day2	100mg/kg	23.73a±1.03	0.00	12.50
	200mg/kg	23.00ab±1.63	"	11.50
	400mg/kg	14.00c±1.08	"	10.50
	Artesunate	12.75d±1.30	"	6.37
	Negative control	45.94e±4.16	"	22.97
Day3	100mg/kg	23.00a±1.08	0.00	11.00
	200mg/kg	19.00ab±1.29	"	9.50
	400mg/kg	14.00c±1.08	"	7.00
	Artesunate	4.37d±0.28	"	2.00
	Negative control	103.38e±4.88	"	52.00

Values with different letters (superscript) are significantly different from each other ($P \leqslant 0.05$) values are in mean ± standard error of four replicate values

aqueous extract – treated groups.

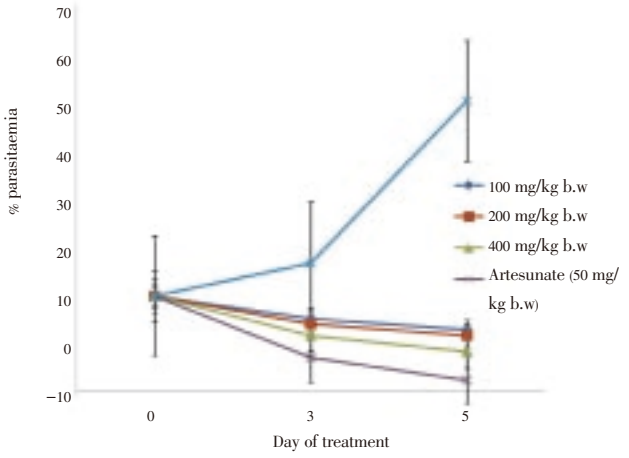

Figure 2. Course of parasitaemia in infected mice treated with methanol extract of *E. camaldulensis* and Artesunate.

3.1.3: Changes in %PCV

The percentage packed cell volumes of all test animals experienced a drastic fall 72 hours post infection but the values for the treated groups increased after 5 days of treatment, while that of the untreated group further decreased (Figures 3 and 4), apparently as a result of continuous haemolysis.

3.1.4: Changes in Weight

The weights of all test animals decreased 72 hours post infection, but this was reversed, for the treated animals after 5 days of treatment. The untreated group experienced a further decrease in weight (Figures 5 and 6).

3.2 Acute toxicity test

Twenty four (24) hours after administration of extracts, no signs of toxicity were observed. All animals survived the study period with no record of death. Initial signs of weakness and difficulty in feeding were reversed on the same day.

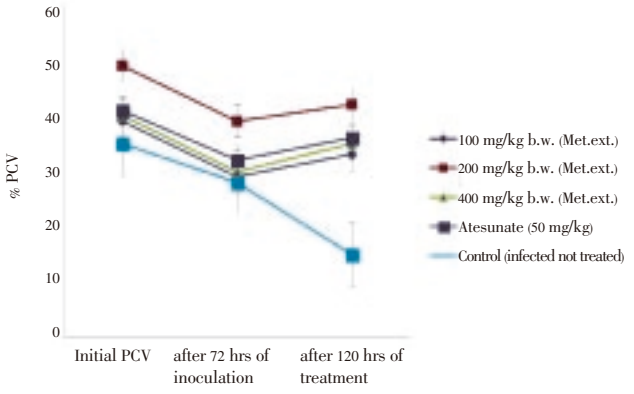

Figure.3: Changes in Percentage Packed Cell Volume (PCV) in infected mice treated with methanol extract of E. camaldulensis

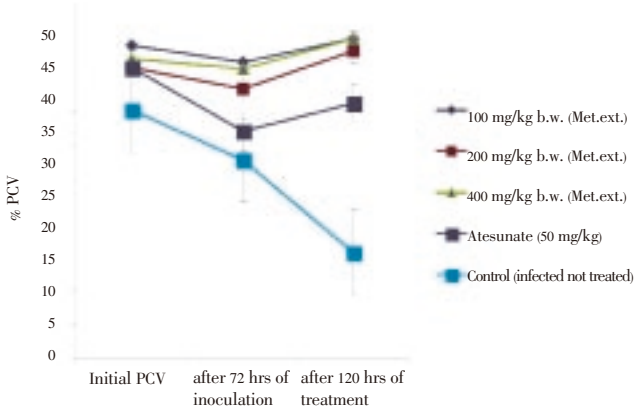

Figure.4: Changes in Percentage Packed Cell Volume (PCV) in infected mice treated with Aqueous extract of E. camaldulensis

Table.2

Level of Parasitemia in infected mice treated with aqueous extract of *E. camaldulensis* leaf

Days of treatment	Dose	Mean Value	P. value	Average % parasitaemia
Day1	100mg/kg	34.50a±1.85	0.997	17.25
	200mg/kg	33.82ab±1.03	"	16.91
	400mg/kg	33.88abc±0.48	"	16.74
	Artesunate	34.13abcd±2.22	"	17.06
	Negative control	34.25abcde±0.85	"	17.12
Day2	100mg/kg	33.63a±0.75	0.00	16.56
	200mg/kg	31.00ab±2.68	"	15.50
	400mg/kg	29.00abc±0.58	"	14.50
	Artesunate	12.75d±1.30	"	6.37
	Negative control	45.94e±4.16	"	22.97
Day3	100mg/kg	31.00a±1.50	0.00	15.50
	200mg/kg	31.00ab±4.51	"	15.00
	400mg/kg	27.00abc±1.16	"	13.50
	Artesunate	4.31d±0.28	"	2.00
	Negative control	103.38e±4.88	"	52.00

Values with different letters (superscript) are significantly different from each other ($P \leqslant 0.05$). Values are in mean ± standard error of four replicate values

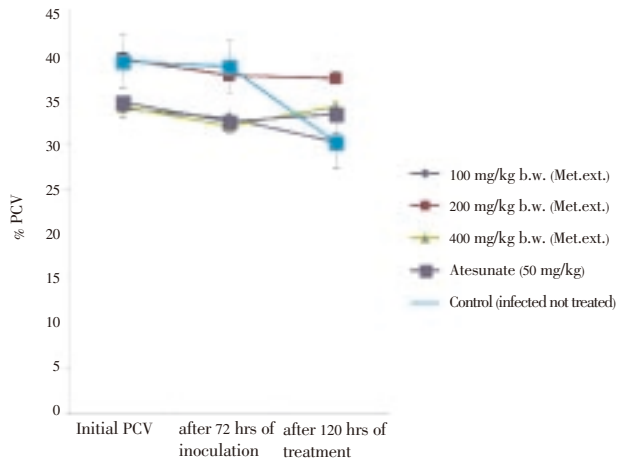

Figure.5: Changes in weight in infected mice treated with methanol extract of *E. camaldulensis*

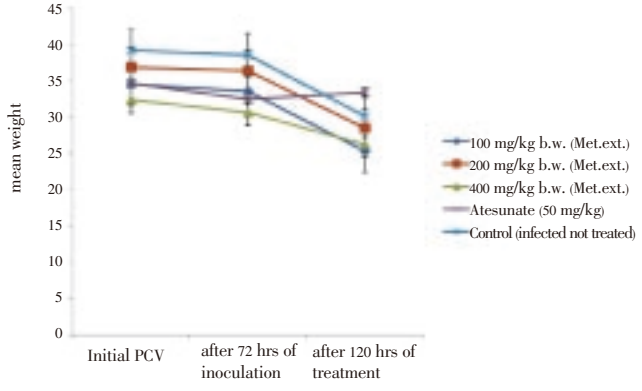

Figure.6: Changes in weight in infected mice treated with Aqueous extract of E. camaldulensis

3.3 Phytochemical screening

The methanol extract was found to contain a high level of terpenes, steroids, fatty acids and low level of tannins and alkaloids; while the aqueous extract contained high levels of terpenes, steroids, saponins and a low level of fatty acids, alkaloids and anthraquinones.

4. Discussion

The present study investigated the antiplasmodial activities of the aqueous and methanol extracts of E. camaldulensis, whose leaves are boiled traditionally in some parts of Nigeria for the treatment of malaria fever. The in vivo results (Figures 1 & 2; Tables 1 & 2) using the two extracts have shown that they are capable of reducing the level of parasites in circulation after 5 days of treatment. Their activities were observed to be dose and time – dependent and significantly higher amounts of the crude extracts were required to elicit such activities when compared to the standard drug, artesunate. However, these extracts, especially the methanol extract can be considered as active antimalarial drugs, since plant extracts are considered active if they demonstrate 50% growth inhibition of the parasite at concentration $\leqslant$ 50 g/ml [19]. Moreover the methanol extract administered at a dose of 400mg/kg bodyweight per day for five days resulted in >50% reduction in % parasitemia, a performance that may likely be improved upon if the crude extract is purified to identify and isolate active substituents. The results also indicate that the use of this plant in traditional medicine for the management of malaria fever is justified, although the need to improve on the methods used traditionally cannot be overemphasized and many medicinal plants of African origin have been shown to demonstrate in vitro antimalarial activities [20], thus necessitating in vivo studies like this one.

Acute toxicity tests with the extracts have also demonstrated their safety because the highest dose used for the screening did not cause death. Interestingly, the highest

dose used to treat parasite – infected mice which elicited anti – malarial activity, was much lower than the highest acute dose.

The presence of plasmodium parasites in the blood stream results in anaemia due to active lysing of Red Blood Cells (RBCs). The results of this study (Figures 3 & 4) show that the initial fall in % PCV of infected mice is reversed after five days of treatment with extracts, thus indicating that the extracts were able to reverse the fall because of their effect on the parasite population.

Malaria fever is a disease that is characterised by loss of appetite which ultimately leads to weight loss. The results in (Figures 5 & 6) are clear indications that the initial weight loss experienced by the test animals was reversed after five days of treatment with the extracts.

The preliminary phytochemical screening of the extracts under investigation showed the presence of terpenes, steroids, fatty acids, tannins and alkaloids in the methanol extract; while the aqueous extract contained terpenes, steroids, saponins, fatty acids, alkaloids and anthraquinones. The antiplasmodial activity exhibited by the extracts may be due to one or more than one group of constituents. Several investigations have been published in the field of antiplasmodials of plant origin related to different bioaditive functional groups classified as: terpenoids; alkaloids; unsaturated fatty acids; volatile oils; and phenolic compounds including flavonoids and quinines [11]. In conclusion, we have demonstrated the antimalarial effects and preliminary phytochemical profiles of extracts obtained from the leaves of *E. camaldulensis*.

Effort will be undertaken to continue biochemical and phytochemical evaluation of the extract in a bid to isolate and identify the active constituents as well as to understand the mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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