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Bioguided investigation of the antimalarial activities of *Trema orientalis* (L.) Blume leaves

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Malaria remains a major public health concern which affects millions of people, particularly in Sub-Saharan Africa. The need for the development of alternate treatment means has become critical because of the emergence of resistance to nearly all antimalarial drugs (Kim and Schneider, 2013). *Trema orientalis* (L.) Blume (Ulmaceae) is used locally for the treatment of malaria. This study was designed to determine the anti-plasmodial activity of the acetone extract of *T. orientalis* and carry out a bio-guided separation of the extract. Acetone extract of *T. orientalis* leaves was investigated for its antimalarial activity in a mouse model of *Plasmodium berghei* using the 4 day suppressive test. Bioguided investigation was carried out by using column chromatographic fractions for *in-vivo* antiplasmodial screening. Preliminary spectroscopic profile of the most active fraction was obtained. Treatment with graded doses (100 to 800 mg/Kg) of acetone extract of *T. orientalis* resulted in significant chemosuppression of parasite growth that ranged from 44.0 to 83.8%. The most active fraction which was identified as M6 showed significant schizontocidal activity ($P < 0.001$). ^{1}H NMR and Infrared spectra data indicated that the most active fraction contained flavonoids. This study justified the folkloric use of *T. orientalis*. Compounds from this plant could be a potential source of antimalarial agents.

Key words: Antimalarial, PCV, *Plasmodium berghei,* anaemia, *Trema orientalis.*

INTRODUCTION

Malaria is a major parasitic disease of the world. This parasitic disease remains a public health concern which affects millions of people, particularly in Tropical African developing countries (Roll Back Malaria-WHO, 2012). The World Health Organization (WHO, 1980) estimates of global incidence of malaria are over 300 million acute cases with approximately two million deaths annually, mostly among young children in sub-Saharan Africa (Rukunga and Simons, 2006; Greenwood and Mutabingwa, 2002). Hence, malaria is Africa's leading cause of under five mortality (20%) and constitutes 10% of the continent's overall disease burden (Marsh, 1998; Panda and Mohapatra, 2004). Medicinal plants which form the backbone of traditional medicine have in the last few decades been subjected to intense pharmacological investigations with a view to discover new lead

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compounds (Makhafola et al., 2012; Samuel et al., 2009).

Trema orientalis is a flowering tree in the Ulmacceae family. It is known by many common names which include pigeon wood in Hawaii and as 'Afefe' in Yoruba land of Nigeria. It has a near universal distribution in tropical and warm temperate parts of the Old World, with a range extending from South Africa, through the Middle East, the Indian subcontinent and Southern China to Southeast Asia and Australia. *T. orientalis* is used locally for the treatment of malaria; other reported uses remedies for asthma and bronchitis (Fatope et al., 2000). A previous study has indicated it has *in-vitro* antimalarial activity (Abiodun et al., 2012).

This study was designed to determine the possible *in vivo* anti-plasmodial activity of the acetone leaf extract of T. *orientalis* against *Plasmodium berghei* infection of Swiss albino mice; and carry out a bio-guided investigation of the extract to identify the most active fraction.

MATERIALS AND METHODS

General methods

Nuclear Magnetic Resonance (NMR) spectrum was obtained on a Bruker 500 MHZ model using Deuterated methanol as solvent and TMS as internal standard. Infrared data was obtained from a Bruker FT-IR spectrophotometer model Vector 22. Silica gel 60 to 120 mesh was used for the Bioguided separation of bioactive extract (All Spectral data were obtained from the Facilities of Indian Institute of Integrative Medicine, Jammu, India).

Plant material

The fresh leaf of *T. orientalis* was collected from its natural habitat at a crop farm at Itaogbolu in Akure North Local Government of Ondo State, Southwestern Nigeria. The plant was identified by the taxonomist of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria and voucher specimen kept at the institute with voucher number assigned. The plant material was dried at room temperature and grounded to powder using a dry electric mill machine. Powdered *T. orientalis* (700 g) was exhaustively extracted by cold maceration in 7 L of acetone (1:10) for 72 h. The mixture was filtered with Whatman's filter paper (No. 1) and the supernatant evaporated with Buchi Rota vapor at 40°C. The solid residue obtained was referred to as the extract. This yielded 41 g (5.85%) of extract which was stored in the refrigerator at 4°C until use. The extract was analyzed using thin layer chromatography (TLC) plates, Merck, Kieselgel 60 F 254 with the following solvent systems: hexane: ethyl acetate (7:3); chloroform: ethyl acetate : formic acid (5:4:1); chloroform: methanol (9:1). Developed plates were sprayed with vanillin-sulphuric acid spray reagent and heated at 110°C for one minute to view the spots.

The dried extract of *T. orientalis* (33, 65, 131, 195 and 260 mg of *T. orientalis*) were dissolved separately in 3.2 mL of distilled water each to produce equivalent doses of 100, 200, 400, 600 and 800 mg/kg/day, respectively. Chloroquine phosphate was obtained from Sigma (USA) and was used as a positive control.

Animal model and parasite

Twenty seven (27) Swiss albino mice weighing between 20 to 22 g were used in this experiment. They were obtained at the Animal house of the Institute of Medical Research and Training (IMRAT), University College Hospital, Ibadan. The mice were used in accordance with the NIH guide for the care and use of Laboratory animals, NIH Publications (Volume 25, Number 28), revised in 1996. Chloroquine sensitive *P. berghei* (ANKA) was obtained from the Malaria Research Laboratory, Institute for Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Oyo state. The animals were distributed into seven groups of 4 mice each. Shortly after inoculation of each mouse by intravenous injection with 1×10^7 parasitized red blood cells, they were administered with 100, 200, 400, 600, 800 mg/kg/day doses of the acetone extract of *T. orientalis* for four consecutive days or chloroquine 10 mg/kg/day for 3 days (Knight and Peters, 1980).

Parasite count was estimated by microscopic examination of Giemsa-stained thin smears prepared from tail snips from day 4, post-infection. Percentage chemosuppression of each dose was then calculated using this formula. Chemo-suppression of parasite growth = 100 - [(mean parasitaemia treated/ mean parasitaemia control) × 100] (Fidock et al., 2004). The ANKA strain of the parasite was used due to its ability to rapidly and consistently grow within the animals. The period of the study was guided by the findings of Anigbogu and Fagbure (1997) who found that the life span of mice inoculated with *P. berghei* is between 7 to 10 days post-inoculation.

Column chromatographic separation of whole extract

Gradient elution was carried out on Silica gel stationary phase with increasing solvent polarity in the following order: hexane: ethyl acetate (90:10), (80:20), (70:30), (60:40), (50:50), (40:60), (30:70), (20:80), (10:90), ethyl acetate 100%, acetone 100% and methanol 100%. A total of 68 fractions (30mL each) were collected and combined on the basis of TLC fingerprint. Six (6) major fractions labeled M1 – M6 were obtained. ^{1}H and infrared spectral data were obtained for the most active fraction [M6] to determine the nature of compound it contained.

Statistical analysis

Data obtained for each group of the experimental mice in the various parameters determined were expressed as the mean ± standard error of the mean (SEM). Statistical comparisons between the groups were made using the one-way analysis of variance (ANOVA) (SAS, 1987). Post Hoc test using Tukey test should be applied after ANOVA to determine the source of difference between fraction treatments. Pairwise comparison between days of the same fraction treatment should be done using paired T test. The level of significant difference between the groups was evaluated at $P < 0.05$ at each level. All above tests (ANOVA, Post Hoc and T tests) need data to be normally distributed.

RESULTS

A paragraph to introduce the results obtained from the treatment groups including the replicates within the same group and the reproducibility of the results would strength the manuscript. This paragraph can be associated with Table 1.

In vivo antimalarial activity of crude extract

The antimalarial activity of the plant was determined in

Table 1. Effects of acetone extract of T. *orientalis* on acute phase of infection early malaria infection.

Extract/drug	Dose	% Parasitaemia	% Suppression
Control (dist. water)	0.2 mL	17.75 ± 3.25	
T. orientalis leaf extract	100 mg/kg	8.28 ± 2.68	59.25
	200 mg/kg	8.3 ± 3.04	60
	400 mg/kg	11.6 ± 4.42	44
	600 mg/kg	9.53 ± 4.16	55.5
	800 mg/kg	3.55 ± 1.82	83.75
Chloroquine	10mg/kg	0.00	100

A significant suppression of % parasitaemia in infected mice when compared with control ($P < 0.001$) was observed. The highest activity was observed at 800 mg/kg with 83.75% suppression.

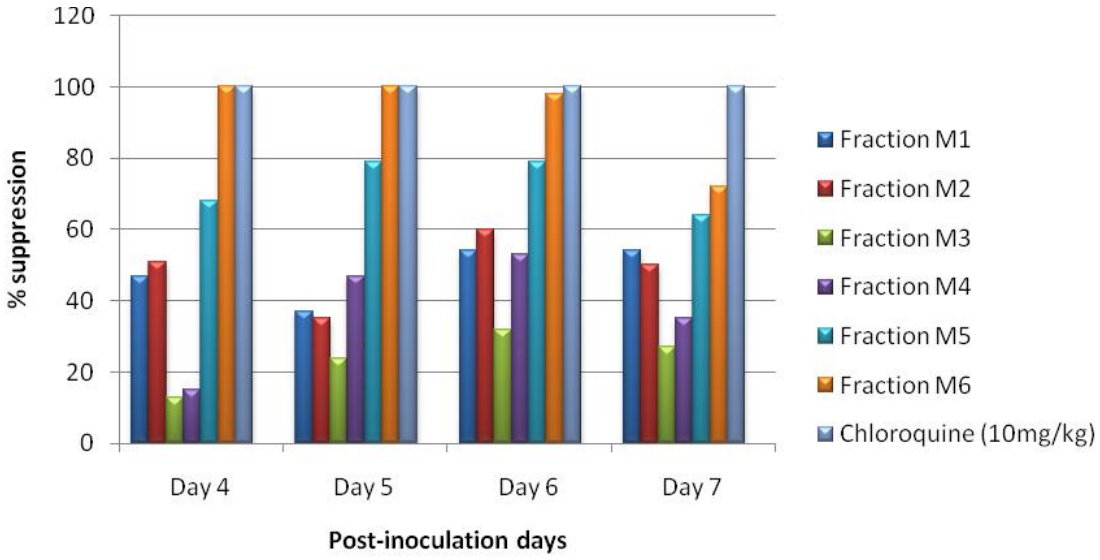

Figure 1. Chemosuppressive effects of chromatographic column fractions percentage schizontocidal activities of column fractions showed M_1 - 48%, M2 - 49%, M3 - 24%, M4 -38%, M5 - 73%, M6 - 93%, while chloroquine had 100%.

vivo using *P. berghei* ANKA strain as the test parasite to inoculate the animals. In these results, when the parasitamea count reduces, the suppression power of the extract is increased and vice versa. Percentage suppression and percentage parasitamea values of the plant extract against the test parasite *P. berghei* for the five test doses compared with the positive control (chloroquine) and negative control (untreated) after the four-day suppressive test are shown in Table 1. The chemosuppression produced by the crude extract was significant ($P < 0.001$) when compared with the negative control. The results show that the parasitamea level in each of the animals for all the test doses reduced considerably compared with the negative control (untreated) group. It also showed that the plant extract had an appreciable chemosuppression activity against the test parasite.

Column separation and in vivo antimalarial activity of fractions

The 68 fractions from the column separation which were combined based on TLC fingerprint produced 6 major fractions; M1, M2, M3, M4, M5 and M6 fractions which gave 1176, 588, 264, 188, 64 and 1072 mg, respectively. Percentage parasitamea and Percentage suppression values of the six fractions against the test parasite *P. berghei* for the 100 mg/kg/day test dose compared with the positive control (chloroquine 10 mg/kg/day) and negative control (untreated) after the four-day suppression test are shown in Figures 1 and 2, respectively. The chemosuppression produced by the six fractions was significant ($P < 0.001$) when compared with the negative control. The results showed that at 100 mg/kg/day experimental dose, fraction M6 showed the

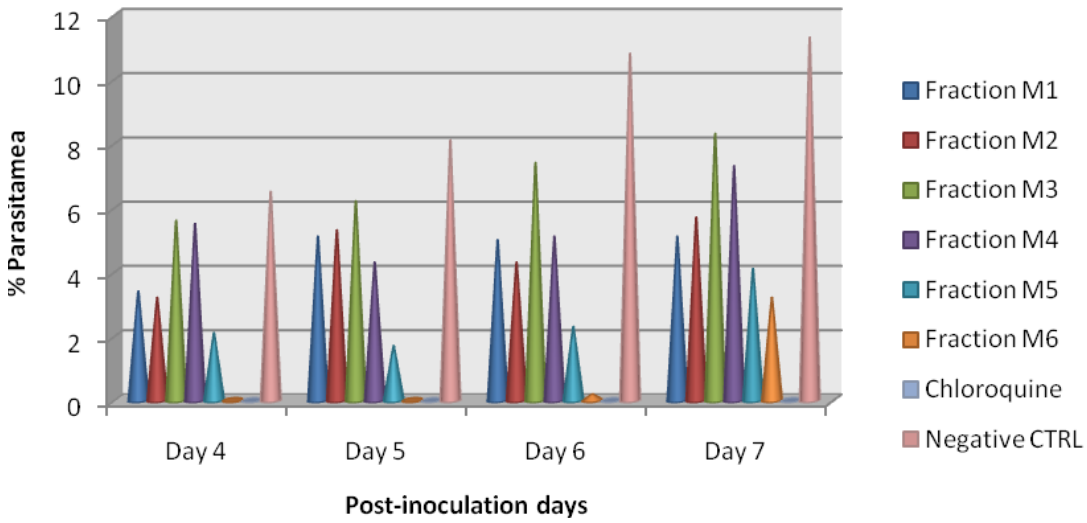

Figure 2. Comparison of the percentage parasitamea of column fractions**.** The bioassay of the tested column chromatographic fractions showed Antiplasmodial activity of Fraction M6.

lowest mean percentage parasitamea (0.9%) and the highest percentage suppression (93%) across the days of the experiment. The positive control drug, chloroquine (10 mg/kg/day) produced a chemosuppression effect of 100%, appreciably close to fraction M6. The chemosuppression produced by fraction M6 on days 4 and 5 of the experiment were 100% which were the same as that of the positive control [chloroquine] in both days. Essentially, the results shown on days 4 and 5 established the schizontocidal activity of fraction M6. Other fractions showed minimal to insignificant schizontocidal activities as shown in Figures 1 and 2. ^{1}H NMR spectra showed downfield signals between 6 and 7 ppm, while infrared spectra indicated the following functional groups -OH, C=O and C-O which are associated with flavonoids.

DISCUSSION

The results from this investigation suggest that the acetone extract of *T. orientalis* has antiplasmodial activities and dosage of 800 mg/kg/day is the most effective dose. However, we observed that treatment at doses of 100 and 200 mg/kg/day were more effective than the dose at 400 mg/kg/day and statistically comparative activity to 600 mg/kg/day. A similar observation has been reported in literature where the methanolic bark extract of *Chrysophyllum albidum* produced a dose independent schizontocidal (chemosuppressive) effect of 74.20 and 62.90% for 1000 and 1500 mg/kg/day, respectively (Adewoye et al., 2010). This observation emphasizes the need for the complete structural elucidation of the bioactive compound(s) with a view to obtaining a clearer picture of its bioactivity profile and mode of action. This is the next target of our group especially because of the high level of siginificance of the activity of this extract ($P < 0.001$). The plant extract exhibited a marked reduction in multiplication of parasites during treatment, indicating that the extract have a direct action on the parasites (Kamei et al., 2000).

Spectra data from our study as shown in Figures 3 and 4 suggested the presence of flavonoid group as the bioactive compound in the extract. Studies by Brandão et al. (1997) on the Antimalarial activities of the ethanolic extract of *Bidens pilosa* identified the flavonoid present in the species as one of components responsible for the antimalarial activity of the plant. de Monbrison et al. (2006) also demonstrated the antimalarial activities of some flavonoid derivatives. The flavonoids group, like the alkaloids, is widely associated with diverse biological activities. Dijoux-Franca et al. (2001) previously isolated three flavonoids from the bark of *T. orientalis*. These flavonoids could possibly be associated with the observed antimalarial activities in the leaf extract we studied.

Previous studies on the *in vitro* antiplasmodial activity of *T. orientalis* indicated it had significant *in vitro* activity (Abiodun et al., 2012; Oyindamola et al., 2011). This current study showed that there is *in vivo* to *in vitro* antiplasmodial activity correlation of this plant.

Conclusion

Extract of *T. orientalis* at all doses used was able to reduce the parasitic load in infected mice. The most active fraction M6 from the extract of *T. orientalis* [M6] also significantly suppressed the parasite load of the infected mice indicating the prospect of this fraction in the

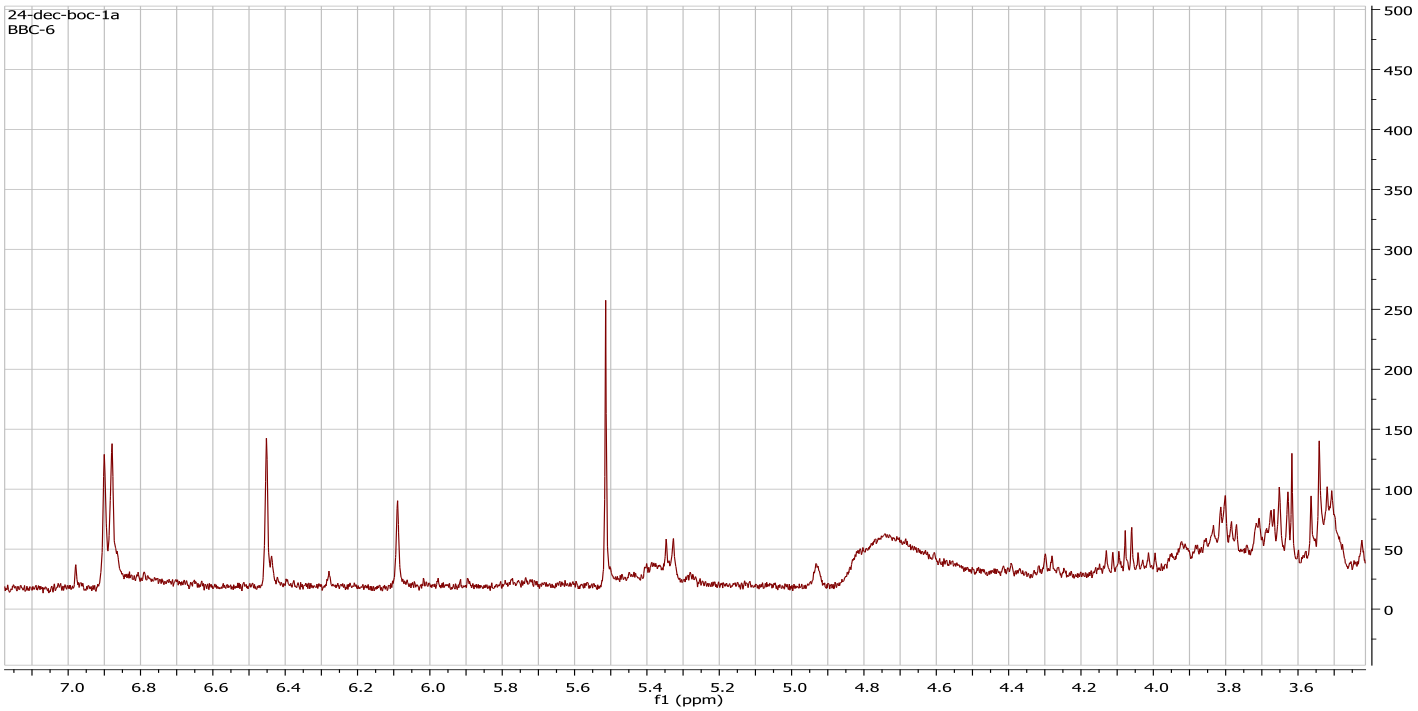

Figure 3. ^{1}H NMR spectrum of compound M6 from *Trema orientalis* leaf extract. ^{1}H NMR spectrum of compound M6 from *Trema orientalis* leaf extract showing downfield signals typical of aromatic systems typical of plant Phenolics such as flavonoids.

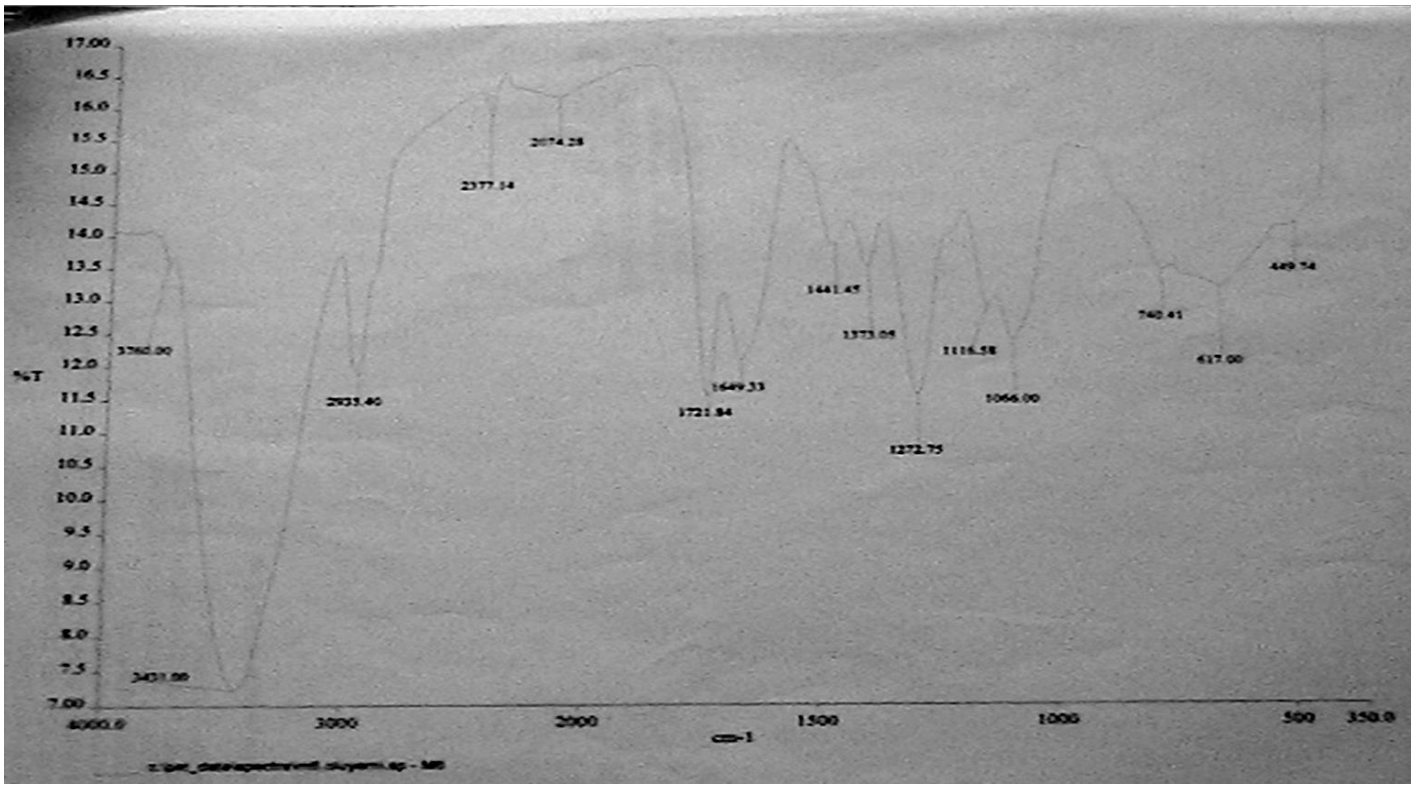

Figure 4. Infrared spectrum of compound M6 from *Trema orientalis* leaf extract. Infrared spectrum of compound M6 from *Trema orientalis* leaf extract showing aromatic and phenolic wave numbers in the isolated compound.

treatment of malaria. ^{1}H and Infrared Spectra indicated the presence of flavonoid in the most active fraction. The *in vitro – in vivo* correlation of the activity of this extract makes it a viable candidate in the search for antimalarial from natural products. Further studies are ongoing to completely elucidate the structure of the bioactive compound(s) in this extract. This study justifies the traditional usage of the plant as antimalarial remedy.

Conflict of interests

The authors did not declare any conflict of interest.

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