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Indigofera oblongifolia protects against trypanosomiasis-induced spleen injury

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ABSTRACT

Background: Trypanosomiasis is a neglected tropical disease, transmitted by blood-sucking insects and can affect humans and animals, depending on the species of Trypanosoma parasite. Trypanosoma has acquired resistance to the majority of drugs used; hence, alternative medicines are required. *Indigofera oblongifolia* leaf extract (IOE) has been shown to treat blood stage malaria. Here, IOE was used to demonstrate its effect on *Trypanosoma evansi*-infected mice.
Methods: Analysis of IOE by gas chromatography–mass spectrometry showed the presence of many active components like flavonoids and phenolics. The mice were divided into three groups as follows: vehicle control, *T. evansi*-infected mice and *T. evansi*-infected-treated mice.
Results: The findings demonstrate a significant effect of IOE treatment on *T. evansi*-infected mice. Parasitemia was decreased by 70%, weight loss was reduced, and splenomegaly was significantly decreased. Additionally, IOE improved the histological architecture of the spleen, as shown by the improved histological injury score post-treatment. Anemia was apparent during the course of infection in *T. evansi*-infected mice; this was reversed upon treatment with IOE to almost the normal level of hemoglobin and erythrocytes. Reduced glutathione and catalase were also ameliorated upon IOE treatment compared to *T. evansi*-infected mice.
Conclusion: Overall, this study shows the ameliorative role of IOE against *T. evansi*-induced spleen injury in mice.

Introduction

Trypanosomiasis is still one of the most dangerous vector-borne diseases, caused by parasitic protozoan trypanosomes. In Africa, this disease related to an annual loss reaching 1.5 billion USD [1]. Trypanosomiasis is widely distributed in tropical and sub-tropical regions and affects several domestic and wild animals [2,3]. The clinical signs involve weakness, rapid weight loss, fever, and anemia [4].

Trypanosoma evansi is an extracellular parasite that survives in blood and causes a severe disease in the veterinary field, known as 'Surra' in Asia and Africa [2,3]. *T. evansi* is transmitted from an infected host to another host by blood-sucking horseflies [2,5].

Like other blood parasites, Trypanosoma has acquired resistance to most of the commonly used drugs to treat the infection, hence the risk of death. Additionally, current medications induce severe side effects [6]. Therefore, the discovery of new agents against trypanosomiasis is required.

The plant *Indigofera oblongifolia* is distributed in many countries across Asia and Africa and is traditionally used against malaria symptoms [7]. Most of the plant parts are used against hepato- and splenomegaly [8]. The aim of this study was to assess the potential effect of *I. oblongifolia* leaf extracts against spleen and liver induced injuries in mice, due to *T. evansi* infection.

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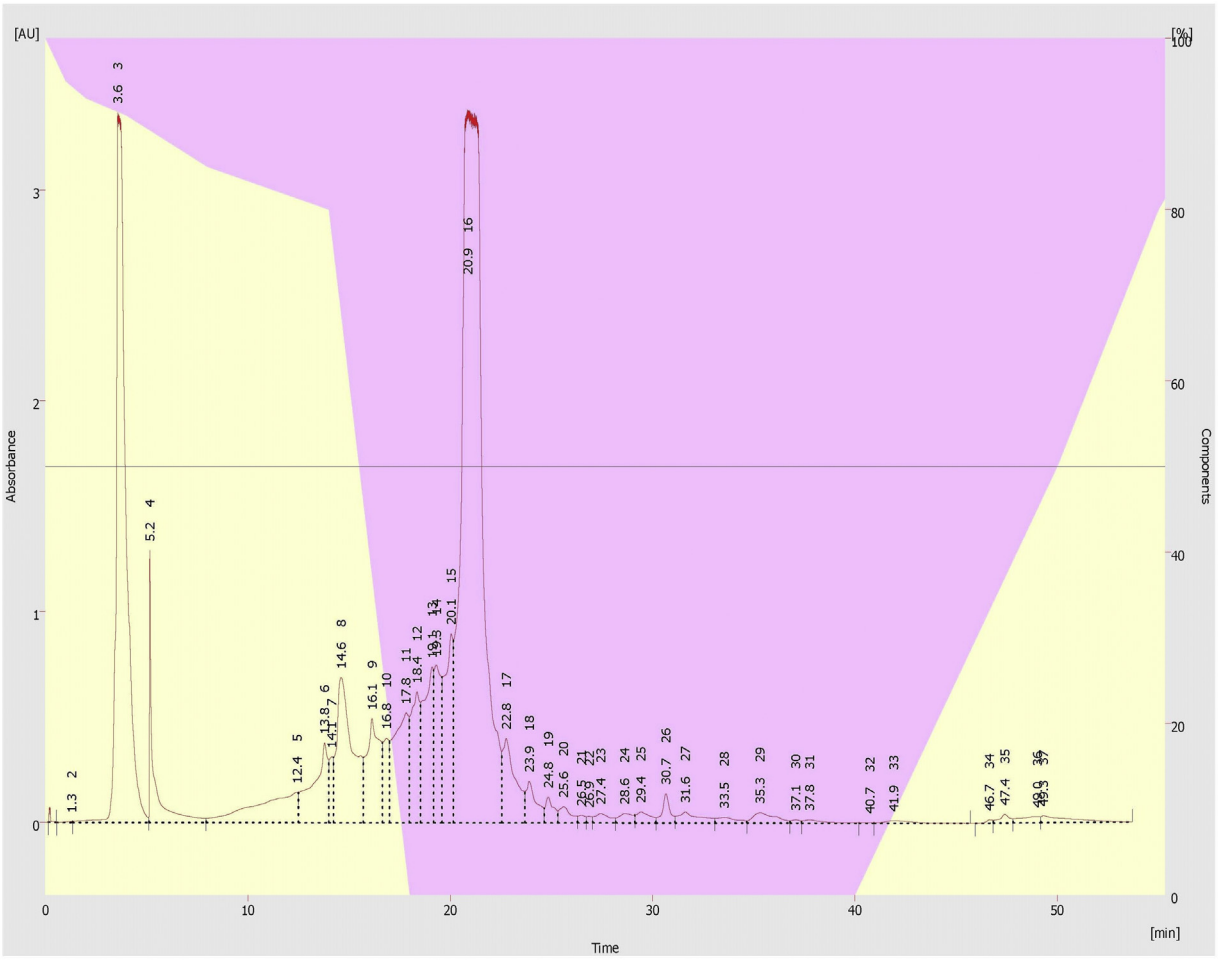

Fig. 1. HPLC chromatogram of the methanolic extract of *I. oblongifolia* leaves showing 39 peaks with retention times ranging from 0.197 min to 49.32 min A mobile phase consisting of mixture of solvent A (0.2% acetic acid) and B (acetonitrile) and employing a gradient elution (from 10:90 to 100:0, *v*/*v*) at a flow rate of 1 mL/min.

Materials and methods

I. oblongifolia *leaf extracts (IOE)*

The acquisition of *I. oblongifolia* leaves was carried out from a region known as Jazan, in Saudi Arabia. The collected leaves were dried at a temperature not exceeding 40 °C, before being ground into powder. Alike the method used by Al-Shaebi et al. [9], extractions were carried out using 70% methanol. Distilled water was used to dissolve the residue.

High-performance liquid chromatography (HPLC) analysis

A centrifugation step for 2 min at 1100 × *g* followed by filtration through a 0.45-μm filter was conducted for all the three IOE replicates. An HPLC apparatus with a diode array detector (DAD) was used. The HPLC column was a ZORBAX Eclipse plus C18 from Agilent Technologies, Palo Alto, CA, USA. Acetic acid (2%; A) and acetonitrile (B) were used for elution. The elution gradient used was as follows: 0 min, 5% B; 2 min, 7% B; 4 min, 9% B; 6 min, 12% B; 8 min, 15% B; 9 min, 16% B; 10 min, 17% B; 11 min, 17.5% B; 12 min, 18% B; 14 min, 20% B; 16 min, 28% B; 18 min, 100% B; 22 min, 100% B; 23 min, 5% B. The initial conditions were sustained for 5 min. Throughout the elution, the flow rate was set at 0.80 ml/min. At 30 °C, separation was carried out. The DAD was programmed to operate at 280 nm.

Animals and infection

Swiss, albino, female mice (9–13 weeks old) were obtained from the animal facility of the Zoology Department, College of Science, King Saud University. *T. evansi* was kindly provided by Prof. Mehlhorn (University of Duesseldorf, Germany). The strain was maintained in mice by weekly transfusions with infected blood. Parasitemia was estimated according to a previous report [10]. In brief, one drop of blood was collected from the tail vein of every *T. evansi*-infected mouse, followed by counting the number of parasites present in at least 10 fields at ×400 magnification power of a light microscope. The study was approved by the ethical committee of the parasitology group at the College of Science, King Saud University for the project RG-198.

Experimental design

Thirty mice were divided into three groups, with ten animals per group. Mice in the first group were considered as a control (non-infected) group, receiving 100 μl distilled water by oral gavage (daily for 4 days). Mice of the second and third groups were intraperitoneally infected with 1×10^3 *T. evansi*. An hour later, the third group was treated according to the method used by Lubbad et al. [7] with 100 mg/kg of *I. oblongifolia* leaf extract for four days, once daily.

Total erythrocyte count and hemoglobin content

Mice were killed by CO_2 asphyxiation for sample collection on day four, post-infection (p.i.). Blood was collected into heparinized tubes from the heart, in order to determine the hemoglobin content and the total erythrocyte count using an automatic counter (VET-530 CA Medonic; Medonic, Stockholm, Sweden).

Weight change and spleen index

Weight changes in the mice were recorded and the spleen index was determined as the ratio of the spleen weight against the weight of the mouse.

Histopathological study

Spleens were fixed in 10% neutral buffered formalin. Samples were then embedded in paraffin and sectioned with a thickness of 5 μm and finally stained with hematoxylin and eosin. According to Giamarellos-Bourboulis et al. [11] we used a semi-quantitative scoring system to evaluate the splenic histological alteration.

Reduced glutathione and catalase

Animal spleens were homogenized in Tris–HCl and sucrose according to the method of Tsakiris et al. [12]. Reduced glutathione in the spleen, was estimated according to the method used by Ellman [13] and reduced chromogen was measured at 405 nm. Catalase was determined according to according to Aebi [14].

Statistical analysis

A statistical package program (SPSS version 17.0) was used to carry out One-way ANOVA analyses and statistical comparisons (Duncan's *t*-test) among mice groups. A result of $P \leq 0.05$ was considered to be significant for all statistical analyses in this study.

Results

The polyphenolic and flavonoid fingerprint for IOE detected at 280 nm is shown in Fig. 1 and Table S1. The HPLC profile of IOE shows 37 peaks with retention times ranging from 0.197 min to 49.32 min. Based on the UV–vis spectral data and their retention time, the IOE has a UV band at 280 nm, characteristic of phenolic and flavonoid compounds, for example vanillic acid, cyanidin and its derivative, luteolin and its derivatives, gallic acid derivatives, caffeic acid, indigo, kaempferol and quercetin derivatives. The identification of these components based on Abdel Moneim [15].

IOE significantly decreased the parasitemia by approximately 70% compared to that in the infection group (Fig. 2). The difference in parasitemia between the infection and the infection-treated animals become clear at day four p.i. with *T. evansi* (Fig. 2).

T. evansi induced significant ($P \leq 0.01$) weight loss in mice (Fig. 3). Treatment of mice with 100 mg/kg IOE significantly decreased the percentage of weight loss due to infection (Fig. 3).

Macroscopically, we detected an increase in spleen size after infection. By measuring the spleen index, it was clear that *T. evansi induced* splenomegaly and the spleen index increased by almost two-fold, compared to the non-infected spleens (Fig. 4). This indicates that the plant extract could reduce the spleen index of the infected animal (Fig. 4).

Microscopic examination of spleen sections demonstrated that infection alters the spleen architecture, with white pulps becoming enlarged and forming together (Fig. 5). After treatment, the spleen structure was improved and white pulps become clearly organized (Fig. 5). By analyzing the spleen sections, it was clear that there was

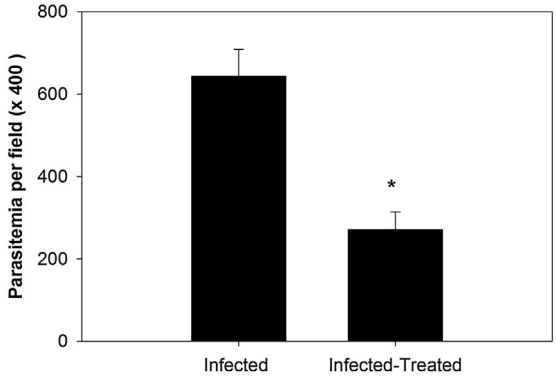

Fig. 2. Parasitemia in mice infected with *T. evansi* and post-treatment with IOE. (*) indicates a significant difference between infected and infected-treated groups at $P < 0.01$.

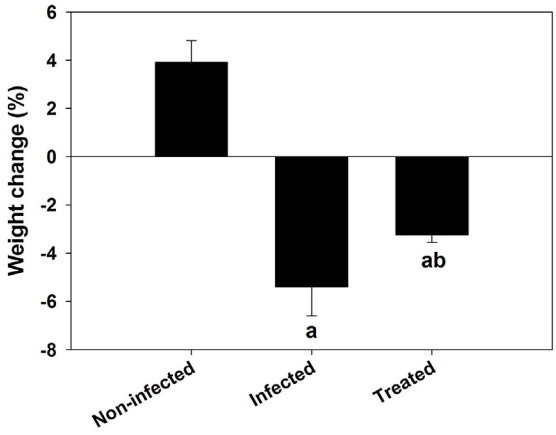

Fig. 3. Percentage of weight change in control, *T. evansi* infected, and infected-treated mice with IOE. Values represent mean ± SD. (a) and (b) indicate the significant difference between control and infected groups at $P < 0.01$, respectively.

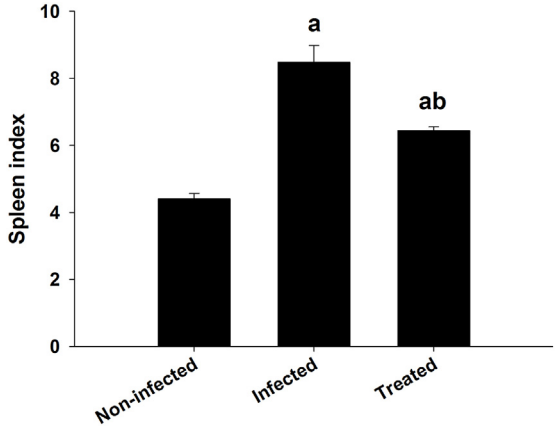

Fig. 4. Spleen indexin control, *T. evansi* infected, and infected-treated mice with IOE. a) and (b) indicate the significant difference between control and infected groups at $P < 0.01$, respectively.

an increase in the histology score in the *T. evansi*-infected animals (Fig. 6), while after treatment with IOE, the score was decreased by approximately 40%.

The decrease in hemoglobin content and erythrocyte count in blood of mice at day four p.i. with *T. evansi* could be diagnosed as anemia. The hemoglobin content of the infected group reached 12.3 ± 0.5 g/dl. In addition, the erythrocyte count of the infected group reached $7.8 \pm 0.1 \times 10^{12}$ erythrocyte/L (Table 1). The extract

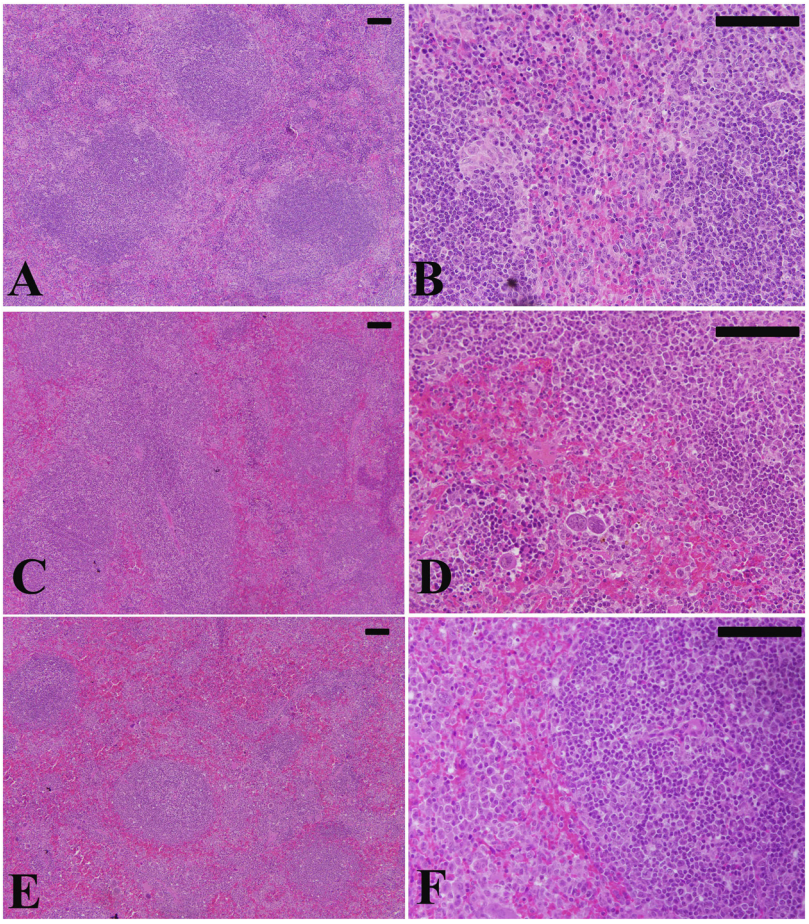

Fig. 5. Induced changes by *I. oblongifolia* leaf extracts in the spleen of mice infected with *Trypanosoma evansi* on day four p.i. Non-infected spleen sections (A and B) appeared with normal architecture. Infected spleens (C and D). Sections display fused white pulps. Spleen of mice treated with 100 mg/kg IOE (E and F) appeared with improved structure. Sections are stained with hematoxylin and eosin. Scale bar = 100 μm.

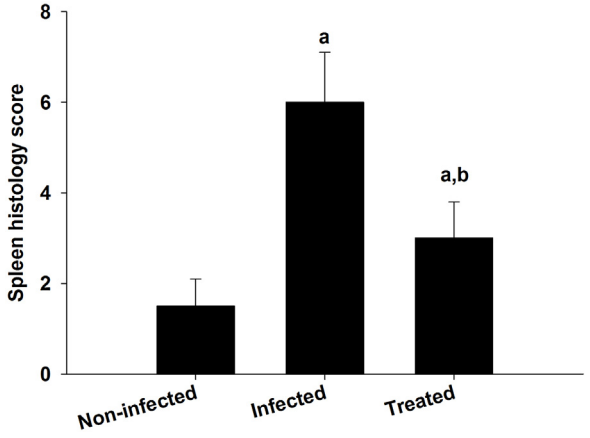

Fig. 6. Spleen histology score in non-infected, *T. evansi* infected and infected-treated mice. Values are means ± SD. (a) Significant change against non-infected group at $P \leq 0.05$. (b) Significant change against infected group at $P \leq 0.05$.

of *I. oblongifolia* can significantly reduce the loss in hemoglobin, as well as the erythrocyte count (Table 1).

Fig. 7 presents the induced change in the reduced glutathione level after infection and treatment of mice with IOE. *T. evansi* was able to decrease the glutathione level in the mice spleen ($P < 0.01$). Treatment of mice with IOE caused an elevation of the reduced glutathione. Also, catalase activity was decreased after infection and increased after treatment of mice with IOE (Fig. 8).

Table 1
I. oblongifolia-induced changes in the number of erythrocytes and the hemoglobin content in mice infected with *T. evansi*.

Groups	Erythrocytes ($\times 10^{12}$/L)	HGB Hemoglobin (g/DL)
Non-infected	9 ± 0.7	14 ± 1
Infected	7.8 ± 0.1[a]	12.3 ± 0.5[a]
Infected-treated	8.6 ± 0.5[b]	14.3 ± 0.5[b]

Values are means ± SD.
[a] Significance against non-infected group at $P \leq 0.01$.
[b] Significance against infected group at $P \leq 0.01$.

Discussion

Trypanosomiasis is still one of the most dangerous diseases, causing severe economic loss when infecting domestic animals. In this study, our aim was to find an effective agent against the causative parasite, Trypanosoma. Consequently, we found that IOE was able to significantly lower the number of trypanosomes (Fig. 2). This protective effect may be due to the presence of IOE active compounds like saponins, phenol, quinines, and coumarin [16].

The induced decrease in mice weight due to infection with *T. evansi* (Fig. 1) was also reported by Desquesnes et al. [17]. The decrease in erythrocyte number, in addition to the decrease in hemoglobin content (Table 1) is an indication for anemia development. Generally, trypanosomiasis is characterized by the development of anemia [18,19]. The induced anemia is due to the destruction of erythrocytes [20]. Shehu et al. [21] reported

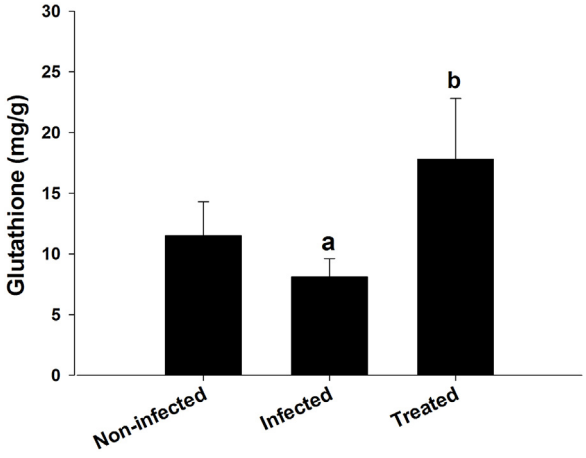

Fig. 7. Change in glutathione level in control, *T. evansi* infected and infected-treated mice spleen. Values are means ± SD. (a) Significant change against non-infected group at $P \leq 0.05$. (b) Significant change against infected group at $P \leq 0.05$.

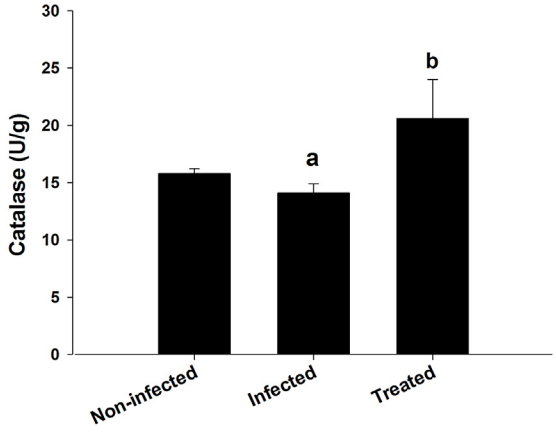

Fig. 8. Change in catalase activity in control, *T. evansi* infected and infected-treated mice spleen. Values are means ± SD. (a) Significant change against non-infected group at $P \leq 0.05$. (b) Significant change against infected group at $P \leq 0.05$.

that neuraminidase enzymes produced by trypanosomes, is the main cause of erythrocyte destruction. In general, the induced anemia occurs concurrent with the increased level of parasitemia [21].

For the parasite to develop, it utilizes glucose and oxygen to grow and multiply, which leads to degenerative changes in the host, as a consequence of the loss of metabolites [22]. Additionally, the parasite releases toxicants that affect the organs and induce cellular damage [22]. This then affects spleen structure and function.

The spleen is considered the most important lymphatic organ among the lymphatic tissues that serve as the first line of defense against Trypanosoma infection. Induced splenomegaly and hyperplasia of spleen cells were also reported by Biswas et al. [23]. Additionally, necrosis was prominent, which caused severe lesions [24]. The increase in spleen index due to infection and induced splenomegaly is a result of disease progression [25].

It is well known that glutathione is an intracellular redox agent playing a vital role in cellular protection against oxidative damage [26]. Netto et al. [27] reported its role as a marker and a reducer of oxidative stress. Also, catalase enzyme acts to decompose hydrogen peroxide into water and oxygen to prevent the induced damage induced by hydrogen peroxide [28]. It was shown that IOE had antioxidant properties, with the potential to improve the induced oxidative damage due to parasitic infection in the spleen [7].

Conclusion

Generally, *I. oblongifolia* leaf extract are reported to be an effective agent against hepatomegaly and splenomegaly [29]. In our study, *I. oblongifolia* extracts were shown to reduce parasitemia induced by *T. evansi* infection and improve the splenic alteration induced by the parasite.

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Competing interest

None declared.

Ethical approval

Not required.

Acknowledgment

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2019.03.005.

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