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Research Article

Anti-Trypanosomal Activity of *Guiera senegalensis* on *Trypanosoma brucei* Infected Mice

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ABSTRACT

Aqueous decoction of *Guiera senegalensis* leaves was studied orally and intraperitoneally for its antitrypanosomal activity on mice infected experimentally with *Trypanosoma brucei brucei*. After a phytochemical screening followed by an acute toxicity study on mice, the extract of plant was administered once daily for 2 days at doses of 60, 120 and 240 mg / kg orally and 15, 30 and 60 mg / kg intraperitoneally after infection. Then, parameters of parasitaemia, packed cell volume (PCV), mean survival time and body weight of the mice treated with the extract were measured and compared with positive (diminazene aceturate) and negative (distilled water) controls for 7 days in a row. Results indicate that the aqueous extract of *G. senegalensis* leaves contains tannins, flavonoids, saponosides, reducing compounds and anthocyanosides, alkaloids and coumarins. LD50 of the extract are 1264.49 mg / kg by oral route and 316.22 mg / kg by intraperitoneal route. The doses of 240 mg / kg by oral route and 15 and 60 mg / kg by intraperitoneal route of aqueous extract showed a mean survival time (5 days) comparable to the positive control. Parasitaemia level increased in all mice tested except in mice treated with diminazene aceturate during the post-infestation period. During this period, PCV and body weight of all mice decreased by both routes of administration. These results of the study show the pharmacological utility of *G. senegalensis* leaves in the control of TAA by herders / pastoralists and suggest continuing further bio-guided studies to isolate the active components of the plant in order to improve their efficiency.

Keywords: *In vivo* test; *Trypanosoma brucei brucei*; *Guiera senegalensis* leaves; phytochemical screening; acute toxicity.

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INTRODUCTION

Parasitic diseases such as African Animal Trypanosomoses (AAT) are one of the impediments to livestock development in sub-Saharan Africa ¹. The fight against TAA includes several strategies including vector control, the use of trypanocidal molecules and the development of vaccines. Vector control with continental programs such as PATTEC (Pan African Tsetse and Trypanosomosis Eradication Campaign) which, in addition to being expensive and laborious, has absolutely not allowed to eradicate tsetse flies like originally planned ^{2,3}. The use of trypanocidal molecules for prophylaxis (isometamidium chloride) or curative

(diminazene aceturate) unfortunately has many cases of trypanosome resistance in more than 18 African countries ^{4, 5, 6}. As for the development of vaccines, this strategy is uncertain in the near future ^{7,8}. In the light of these findings, new researches on the study of the trypanocidal activity of certain medicinal plants commonly used by herders/pastoralists are increasingly being explored ⁹.

In Burkina Faso, rural population often uses ancestral knowledge of medicinal plants to treat diseases of humans and animals ^{10, 11}. Recent studies conducted in the country's Gaongho Pastoral Zone have shown that farmers extensively use plants to treat animal diseases such as AAT ¹².

Among these plant species, *Guiera senegalensis* leaves are commonly used and its trypanocidal efficacy *in vitro* on *Trypanosoma brucei brucei* has been proven in a previous study¹³. Also, the present study was performed to evaluate the anti-trypanosomal activity *in vivo* of *G. senegalensis* leaves according to the procedure of the herders against *T. b. brucei* infected mice.

MATERIAL AND METHODS

Plant material and preparation

The leaves of the *G. senegalensis* plant were collected in the pastoral area of Gaongho. The specimen of the plant was identified (Voucher specimen number 5952) at the herbarium of "Centre National de la Recherche Scientifique et Technologique" in Burkina Faso.

The leaves of the plant were dried and powdered to prepare a decoction aqueous extract in accordance with the herders' procedure¹³. Briefly, 200 g of the sample of the plant material was placed in a 2000 ml beaker equipped with a lid in which a volume of 2000 ml of distilled water was added. The mixture was homogenized with a glass rod and then boiled on a hot plate for 1 hour (mechanical stirring with a glass rod was made from time to time). After, the extract was warmed and then filtered by pressing on a fine nylon cloth. Obtained filtrate was then centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and concentrated at a temperature of 45°C in a ventilated oven. The concentrated decoction obtained was transferred to freezer jars for freeze-drying in order to carry out phytochemical screening and biological tests.

Experimental animals

Healthy female NMRI albino mice, weighing 25-30 gm and age of 8 weeks, from the "Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES)" were used. Animals were kept in polypropylene cages (6 animals per cage) under the same conditions of temperature (25 °C), relative humidity of 70-80% and 12:12 h dark / light cycle with free access to standard diet and tap water *ad libitum*.

Trypanosome parasite

The strain of *T. b. brucei* (reference Farako-ba 80 / CRTA / 01) was provided by the CIRDES where it is stored in nitrogen liquid (-180 °C). This strain was used to conduct the test of the extract antitrypanosomal activity on mice.

Phytochemical Screening

The extract was subjected to phytochemical screening for the presence of active principles using standard screening method of Ciulei¹⁴ (1982).

Biological tests

(i) Determination of the acute lethal toxicity

Determination of the acute lethal toxicity of aqueous extract was carried out using oral and intraperitoneal routes according to the method described by Lorke¹⁵. In the first phase of the method, nine mice fasted for 12 hours before were randomly divided into three groups of three mice each. Mice from each group received 10, 100 and 1000 mg / kg body weight of the aqueous extract using syringe gavage for oral route and injection for intraperitoneal route. In the second phase of the method, doses of 1600, 2900, and 5000 mg / kg of aqueous decoction were administered to nine mice at the rate of three mice per dose and per route. In parallel, a fourth group of three mice receiving only distilled water was used as a control group in each phase. After

administration of the extracts, all mice were kept under observation to determine the signs of toxicity ie all behavioral, neurological or physical changes at each level. These signs were recorded at 4 hours and 24 hours after treatment of the mice with particular attention on the first 4 hours. At the end of the 24-hour follow-up, lethal doses 50 (LD₅₀) were evaluated according to the two routes of administration applied.

(ii) Antitrypanosomal activity Test

Antitrypanosomal activity test of the aqueous decoction of the leaves of *G. senegalensis* was carried out on mice subjected to two routes of administration (oral and intraperitoneal). For each route of administration, three groups of 6 mice were constituted and submitted to three increasing doses. These doses were obtained from the acute toxicity test which permitted to determine the lethal dose 50 (LD₅₀) of aqueous extract according to each applied administration route (dose 1: [dose 2] / 2, dose 2: [LD₅₀] / 10 and dose 3: [LD 50] x 2). In parallel, two other control groups of 6 mice each were used for comparisons: negative control group (distilled water) and a positive control group (Veriben : diminazene aceturate). The mice of the different groups were distributed in cages for an adaptation period of 7 days (day 7: d₇) under an average temperature of 25 °C before being infected on the eighth day (day 8: d₈) with *T. b. brucei* intraperitoneally. This infection was made with 0.2 mL of infected mice blood (10⁴ trypanosomes / mL). The treatments of the mice were made on the 3rd day post-infection (day 10: d₁₀) when the parasitaemia peak was reached (10⁸ trypanosomes per ml of blood). The treatments were applied for two days in a row (day 10 and day 11: d₁₀ and d₁₁) with the three increasing doses of the extracts. The positive control group was treated at one time (day 10: d₁₀) according to the manufacturer's procedure and the negative control received no treatment. The Antitrypanosomal activity of extract was evaluated using parameters such as parasitaemia, packed cell volume (PCV), body weight and mean survival time of mice.

Determination of parasitemia

The parasitemia level for each mouse was performed daily (d₈ to d₁₅) by microscopic examination of a drop of blood obtained from the tail tip in triplicates¹⁶. The fresh blood was placed between slide and coverslip and observed under a microscope at 400X.

Determination of PCV

PCV was measured every two days (d₇, d₉, d₁₁, d₁₃ and d₁₅) to indirectly determine the degree of anemia of the mice treated with aqueous extract compared to the control groups. For this, the blood was collected from the tail of each mouse in heparinized capillary tubes which were subjected to centrifugation at 12500 rpm for 5 minutes for a good separation of the constituents. The PCV reading was made using a haematocrit reader expressed in percentage.

Body weight

Body weight of each mouse per group was recorded daily from d₇ to d₁₅. For this, the mice were weighed individually.

Mean survival time

This parameter was calculated for each mouse in the treated with plant extract and controls groups throughout the follow-up period by differentiating between the day the mouse dies and the day of treatment.

Statistical analysis

For the calculation of the LD₅₀, the formula was applied : $LD_{50} = \sqrt{(D_0 \times D_{100})}$ where D₀ is the highest dose that causes no mortality and D₁₀₀ is the lowest dose that produces 100% mortality¹⁵. The results of the phytochemical screening were noted - (absence) or + (presence). For antitrypanosomal activity test, data obtained were expressed as mean ± standard deviation of mean of measured parameters and subjected to one-way analysis followed by Tukey's test at 5% for comparison of the results obtained from different groups.

RESULTS

Determination of the LD50

The results of the acute toxicity study of extract are presented in Table 1. The intraperitoneal route presented mice mortality at the dose of 1000 mg / kg in phase 1. In contrast, the oral route induced mortality of the mice at the dose of 1600 mg / kg PC in phase 2. According to formula of Lorke¹⁵ (1983), the lethal doses 50 (LD₅₀) of the extract tested are 1264.49 mg / kg by oral route and 316.22 mg / kg by intraperitoneal route.

Table 1: Acute toxicity effect of *G. senegalensis* leaves aqueous extract administrated orally and intraperitoneal route in mice.

Phases	Doses (mg/kg)	Number of mice used	Mortality number	
			Orally route	Intraperitoneal route
Phase 1	10	03	00	00
	100	03	00	00
	1 000	03	00	03
Control	Distilled water	03	00	00
Phase 2	1 600	03	03	03
	2 900	03	03	03
	5 000	03	03	03

Phytochemical Screening

The phytochemical profile of the extract revealed the presence of phenolic compounds (tannins), saponosides, alkaloids, anthocyanosides, reducing compounds, steroid glycosides, triterpenes, carbohydrates, flavone glycosides and coumarins (Table 2).

Table 2: Phytochemical profile of the aqueous decoction of *G. senegalensis*

Chemical groups	decoction of <i>G. senegalensis</i>
Polyphenols (tannins)	++
saponosides	++
Alkaloids salts	++
Reducing compounds	++
carbohydrates	+
anthocyanosides	++
Steroid glycosides	++
Triterpene glycosides	++
Flavone Glycosides	+
Anthracénosides	nd
Cardiac glycosides	nd
Coumarines and derivatives	+

++ = abundant; + = not abundant; nd = not detected

In vivo test

(i) Mean survival time

Figure 1 show the mean survival time of mice in all groups after the application of treatments compared to the control groups. The mean survival time of the negative control group (distilled water) was one day after the application of the treatment. In contrast, there was an increase in the mean survival time (5 days) of mice in the groups treated with the plant extract at the doses of 240 mg / kg orally and 15 and 60 mg / kg intraperitoneally and the positive control group (Veriben : Diminazene aceturate).

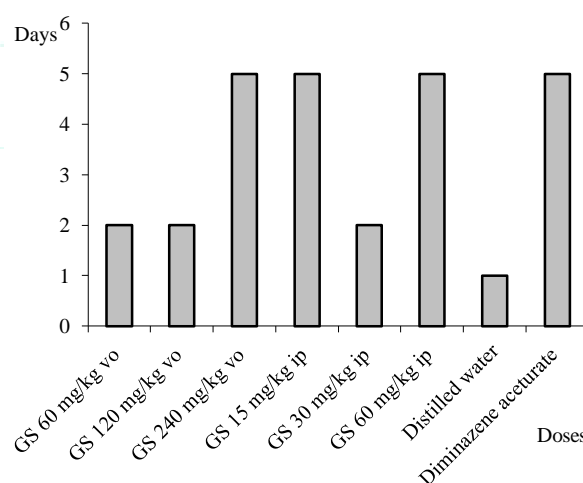


Figure 1: Mean survival time of mice after the treatment application in all groups during the study (GS: *G. senegalensis* ; vo : oral route ; ip : intraperitoneal route).

(ii) Parasitemia Level

Parasitemia levels (in log number / mL) in all groups of mice are presented in Figure 2. The mice were parasitized as early as the second day after infection (d₉) and all died at d₁₁ in the negative control group (distilled water). The mice were parasitized as early as the second day after infection (d₉) and all died on d₁₁ in the negative control group. The number of

trypanosomes in the blood of mice in all study groups increased over time except in the positive control group where trypanosomes died on the second day of diminazene aceturate administration (d₁₁). The degree of parasitaemia between the groups treated with the extract at a dose of 240 mg / kg orally and those treated with the doses of 15 mg / kg and 60 mg / kg intraperitoneally was not significantly different (p > 0.05) during the post-treatment period.

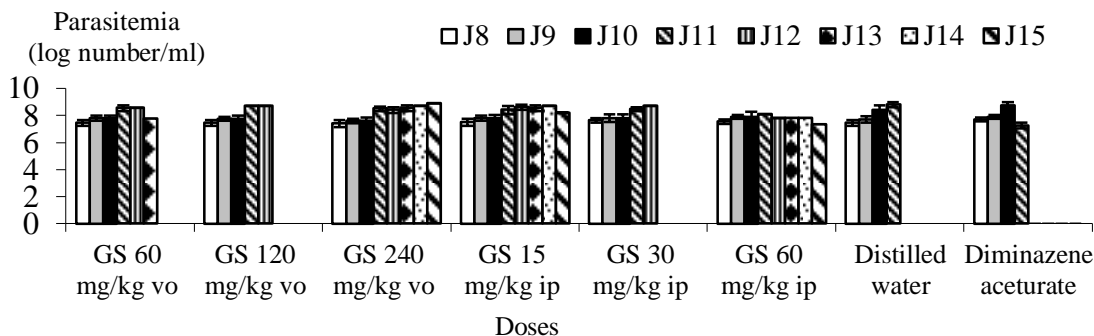


Figure 2: Parasitaemia levels of mice after the treatment application in all groups during the study (GS: *G. senegalensis* ; vo : oral route ; ip : intraperitoneal route) .

(iii) Packed cell volume

Figure 3 shows the PCV levels of mice in the tested groups. PCV means after the infection of mice decreased from d₇ to d₁₅ in all groups. These PCV means of mice group treated

with diminazene acetate were higher (p<0.05) than those of the mice treated with extract at the doses of 240 mg / kg orally and 15 and 60 mg / kg intraperitoneally from d₁₃ to d₁₅.

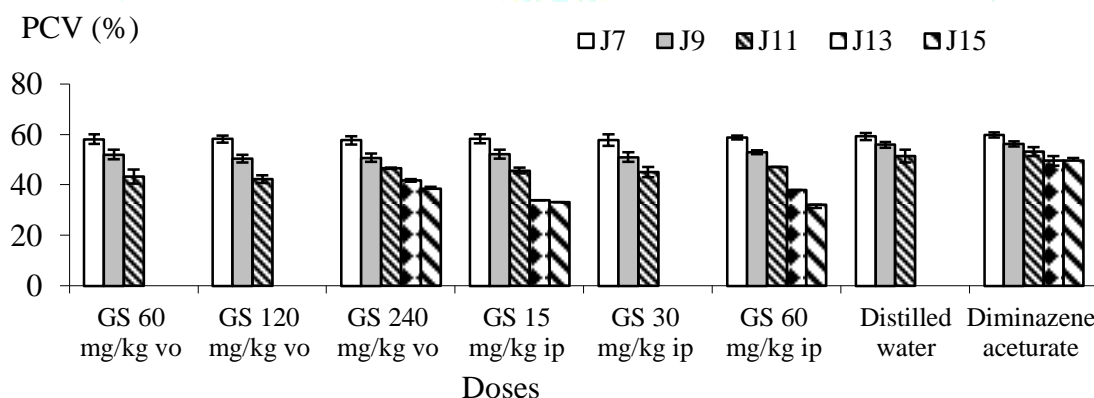


Figure 3: PCV levels of mice after the treatment application in all groups during the study (GS: *G. senegalensis* ; vo : oral route ; ip : intraperitoneal route).

(iv) Body weight change

Body weight change of mice at d₇ to d₁₅ of experiment is reported in Figure 4. After infection with *T. b. brucei*, a significant weight decrease (p<0.05) from d₈ to d₁₅ in experimental mice was observed in all groups. This weight loss was more marked (p<0.05) in the treated groups with

oral doses of 240 mg / kg (10.08%) and 60 mg / kg (7.61%) by the oral route. intraperitoneal compared with the groups treated with diminazene acetate (4.68%) and 15 mg (4.93%) intraperitoneally. With mice treated with diminazene acetate, the weight loss was followed by a weight recovery phase starting on d₁₁.

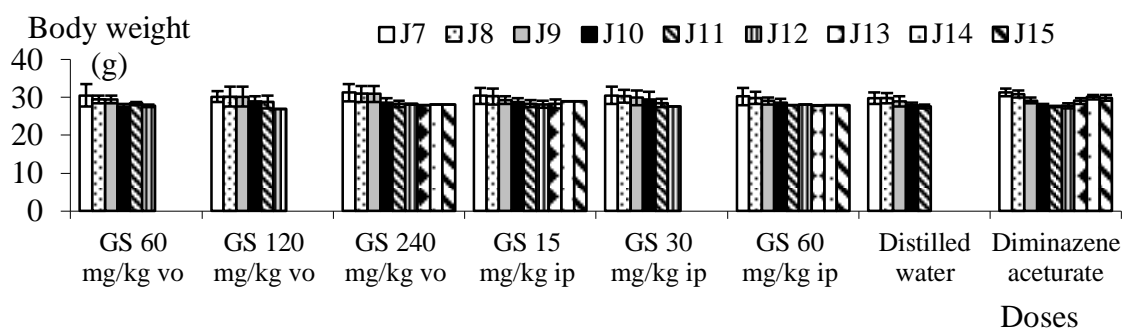


Figure 4: Body weights of mice in mice after the treatment application in all groups during the study (GS: *G. senegalensis* ; vo : oral route ; ip : intraperitoneal route)

DISCUSSION

In this study, the efficacy of aqueous decoction of *G. senegalensis* leaves as trypanocidal in mice experimentally infected with a *T. brucei brucei* strain was tested. This approach aims to explore veterinary ethnomedicine practiced by farmers in rural areas of Africa in the treatment of animal diseases¹⁷. To improve these practices, the study of the toxicity of natural drugs used is very important to determine their safety.

The acute toxicity results of aqueous decoction of *G. senegalensis* leaves in mice have a dose-response effect depending on the administration routes. The LD50 of the extract was 1264.49 mg / kg orally and 316.22 mg / kg intraperitoneally. This difference of the LD50 between the two routes of administration corroborates the observations made by Lahlou et al.¹⁸ and Amein et al.¹⁹ with the aqueous extract of *Tanacetum vulgare* and thymoquinone in mice. The LD50 analysis obtained in our study revealed that the aqueous leaf extract of *G. senegalensis* is slightly toxic by oral route and moderately toxic by intraperitoneal (IP) route according to the toxicity scale of Hodge and Sterner²⁰.

Similar results were obtained with the hydro-methanolic root extract of *G. senegalensis* and the infusion of *Cassia sieberiana* root bark orally by Shettima et al.²¹ and Ajayi et al.²² respectively. By intraperitoneal route, the results obtained in our study are comparable to those of Fané²³ with the aqueous macerate of *C. sieberiana* root bark in mice, although the two methods of preparation are different. The low toxicity of *G. senegalensis* extract orally in mice confirms its usual use by herders/pastoralists and traditional healers in the treatment of various human and animal diseases, including TAA¹².

Phytochemical screening of the leaf extract of *G. senegalensis* revealed several chemical groups with recognized pharmacological activities. These groups are tannins, saponosides, reducing compounds, carbohydrates, anthocyanosides, steroid glycosides, triterpenes, alkaloid salts and coumarins and derivatives. These results are in agreement with those of Kouamé et al.²⁴ and Yagana et al.²⁵ who found tannins, saponosides, carbohydrates, terpenoids, flavonoids and alkaloids in the *G. senegalensis* leaves. Similarly, the presence of coumarins and derivatives obtained in our study confirms the observations of Soulama et al.²⁶ who report that natural coumarins are secondary metabolites synthesized by plant leaves. All these chemical groups contained in the extract would have the power to act on one or more target sites associated with a physiological process²⁷ and to interfere with the redox balance of parasites acting on the

respiratory chain or cellular defenses against oxidative stress that partially eliminate trypanosomes^{27,28}.

Indeed, flavonoids inhibit the growth of trypanosomes and act on trypomastigote forms found in the bloodstream of mammals^{29,30}. Similarly, flavonoids from natural plants act as free radicals and metal chelators that inhibit lipid peroxidation and exhibit various physiological activities, including antihypertensives and anti-arthritic activities³¹. Alkaloids reduce the growth of trypanosomes by intercalating deoxyribonucleic acid (DNA) with trypanosomes³² and inhibiting protein synthesis²⁹. The anti-trypanosome activity of the phenolic compounds is the inhibition of the alternative trypanosome oxidase enzyme³³. According to Taylor et al.³⁴—one of the molecular actions of tannins is to complex proteins through so-called non-specific forces such as hydrogen bonding, the hydrophobic effect as well as the covalent bond formation. For terpenes, the mechanisms of trypanocidal activity include the formation of aldehyde-thiol adducts with sulfur-containing components, thereby decreasing the buffering of agents that can create oxidative stress in the cells³⁵ and the oxidation of glutathione and pyruvic acids³⁶.

After infection of the mice on day 8, a high parasitemia (10^8 trypanosomes per ml of blood) was observed in all the groups on day 10 followed by mouse mortality between 2 and 7 days. The pre-patent period (2-7 days) of *T. b. brucei* in the study is different from that (7-11 days) obtained by Bengaly et al.³⁷ on mice infected with *Trypanosoma congolense* at a dose of 10^5 trypanosomes/mL of blood. The duration of the pre-patent period could then be thought to depend on the parasite strain and the immune status of the infected host, although *T. congolense* generally has a longer pre-patent period than *T. brucei*. Nevertheless, the survival time of the mice in the study is close to that (5 to 7 days) of Kelly et al.³⁸ who infected CDI strain mice with *T. b. brucei* at the dose of 105 trypanosomes (strain 427). Intraperitoneally, an average survival time of 5 days was observed with two doses (15 and 60 mg / kg) of the extract tested whereas this survival time was only reached with the dose of 240 mg / Kg orally. This shows a clear efficiency of the intraperitoneal route compared to the oral route. These results are in phase with those obtained by Benoit-Vical et al.³⁹ evaluating *Chrozophora senegalensis* in the treatment of malaria on mice. According to these authors, this difference could be due to the low bioavailability of the extract administered orally. In fact, the average survival times of the mice infected and treated with the doses of 240 mg / kg oral and those of 15 and 60 mg / kg by intraperitoneal route could be attributed to the activity of the chemical components that the extract contains. This belief is supported by previous studies that

reveal that polyphenols have the ability to increase the survival of mice infected with *T. b. brucei* ^{40, 41}. Similarly, Mergia et al. ³⁴ revealed that administration of the methanolic extract of *Clusia abyssinica* resulted in a survival time of 39.20 ± 0.37 days in mice infected with *Trypanosoma congolense*. The survival time of the mice despite increasing parasitemia over time in the study suggests that administration of the extract does not completely eliminate trypanosomes from the blood of infected mice during the study. Several works ^{42, 43, 44} have led to similar observations and concluded that a high parasite load could mask the effectiveness of the crude extracts of the plant ⁴⁵. This could be due to the enzymatic inactivation of the phytochemicals that these extracts contain ⁴⁶. On the other hand, the pharmacokinetics of the phytochemicals of the tested extract, including their rapid elimination, may also explain the low in vivo efficacy of the decoction ⁴⁷. These hypotheses reinforce the observations made because the trypanocidal efficacy *in vitro* of the aqueous extract of the plant has been proved previously on *T. b. brucei* with increasing doses of 25, 50 and 100 mg / ml by Zongo et al. ¹³. Similar finding is made by Abedo et al. ⁴⁸ evaluating the trypanosomal activity of methanolic extract of stem bark *Gongorenia latifolium* on mice infected with *Trypanosoma congolense* in Nigeria.

After infection of the mice, the PCV level decreased continuously. This could be explained by hemolysis of red blood cells associated with increased parasitemia. Indeed, trypanosome infections cause an increase in the sensitivity of the red blood cell membrane to oxidative damage. Oxygen-generated oxygen reactions released by trypanosomes can also damage red cell membranes and cause haemolysis ⁴⁹. The effect of the extract in anemia would be to reduce parasite load while neutralizing the toxic metabolites produced by trypanosomes or by trapping free radicals associated with trypanosomes that could be attributed to the secondary metabolites mentioned above ^{45, 50, 41}.

Our results also showed a decrease in mouse weights since their infection with *T. b. brucei*. This is fully in line with the findings of Alli et al. ⁴¹ (2011) who showed that *T. b. brucei* was associated with weight loss. This decrease in body weight could also be explained by the drop in the PCV level associated with a high parasitemia level followed by a decrease in appetite. This results in consumption of fat stores and severe degenerative changes in muscle cells and other tissues in the infested host. In addition, there is increased degradation of proteins in the muscles leading to atrophic degeneration. Decreased supply of oxygen can cause anemia and this is also an important factor ^{51, 52}.

CONCLUSION

The results of this study showed that the decoction of *G. senegalensis* leaves leads to an average survival time of the treated mice comparable to those of the positive control group although they are parasitized. This is probably related to the metabolite substances that the extract contains. Therefore, leaves of *G. senegalensis* could be considered as a potential source for the search for new drugs against TAA. For this purpose, additional studies could be conducted to isolate the active components of the extract to improve the plant anti-trypanosomal activity.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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