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Influence of Graded Dose of *Moringa oleifera* Seed Extract Administered Orally on Testicular Pathology, Gonadal and Extra Gonadal Sperm Reserves of Wistar Rats Experimentally Infected with *Trypanosoma brucei brucei*

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Abstract

BACKGROUND: *Moringa oleifera* seeds are known for their high protein and vitamin content. Antioxidants are abundant in these seeds. Aqueous extraction was done. After that, an acute toxicity test was performed.

OBJECTIVES: The purpose of the study was to see how a graded dose of *M. oleifera* aqueous seed extracts altered testicular pathology, gonadal and extragonadal sperm reserves in Wistar rats infected with *Trypanosoma brucei brucei*.

METHODS: The rats were A, B, C, D, and E were randomly assigned to five groups with group E serving as the control group. The rats in Groups A to D were inoculated intra-peritoneal with 1× 106 virulent *T. brucei brucei*, and they were held for one week to demonstrate clinical signs before starting the extract therapy. Every day at 10:00 a.m., the rats were given treatment for five weeks with (75, 100, 125, and 150) mg/kg of *M. oleifera* aqueous seed extract for groups A, B, C, and D respectively. While the control group E received 0.5 mL/kg of water. For hematological indices, blood samples were collected every Monday between 10:00 and 11:00 a.m. All of the rats were humanely sacrificed at the end of the six-week experiment, and their gonadal and extragonadal sperm stores were collected, tested, and processed for histopathology.

RESULTS: After treatment, the rats' gonadal and extragonadal sperm reserves (groups A to E) showed a substantial increase (213±1.1a; 221±2.1; 250±0.0c; 259±2.6d; 295±2.6e) × 10^6 and (115±1.1; 160±2.1; 153±0.0; 167±2.6; 120±0.6) × 10^6 respectively, compared to control group at $P < 0.05$ level. Sperm concentration of the right epididymis (60.0±1.1a; 90.2±2.1b; 96.5±0.0c; 98.7±2.6d; 69.4±0.6e) × 10^6 were significantly higher compare to the left epididymis (55.0±1.1; 69.8±2.1; 56.5±0.0; 68.3±2.6; 50.6±0.6) × 10^6. The PCV (%) and WBC (103/µL) levels in groups A, B, and C were significantly greater following infection than that in group E. Infection with *T. brucei* at weeks 2 and 3 shows poor semen characteristics, thereafter the semen quality has improved.

CONCLUSIONS: *Moringa oleifera* aqueous seeds extract has drastically abridged the impact of trypanosomosis and enhanced the semen quality of the experimental rats.

KEYWORDS: Male, Nutrition, Protozoa, Reproduction, Supplement

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Introduction

Animal trypanosomosis is one of the major factors limiting the development of the African livestock industry. (Lemu *et al*., 2019). Trypanosomosis has continued to cause havoc on animal production in developing countries, both economically and socially. (Abdullahi *et al*., 2018; Odeniran *et al*., 2018). African trypanosomosis is a parasitic disease caused by a protozoan parasite of the Trypanosoma genus. (Opaluwa *et al*., 2015). Tsetse flies transmit this organism, which is a major cause of livestock morbidity and mortality (Abubakar et al., 2016). This scourge is still a concern for African scientists, who are working on a roadmap to develop prevention and treatment options (Karshima *et al*., 2016). Trypanosomosis, also known as sleeping sickness or nagana in humans and animals, is caused by African trypanosomes. The trypanosome organisms that affect people and their pets have been classified into two categories (Odeyemi *et al*., 2015) The haematinic group (*Trypanosoma congolense*, *T. vivax*) is contained in plasma, while the tissue invading group (*Trypanosoma brucei*, *T. evansi*, *T. gambiense*, *T. rhodesiense*, and *T. equiperdum*) is found in extravascular and intravascular spaces (Krinsky, 2019; Lemu *et al*., 2019).

As recorded in Yankasa rams by Iliyasu *et al*. (2014) parasitic disease is characterized by damage to central metabolic organs such as the liver and reproductive organ degeneration. Oxidative stress caused by trypanosome and macrophage behaviors is one of the main factors involved in disease pathogenesis. (Lokugamage *et al*., 2020). Oxidative stress has been observed in rabbits infected with different trypanosome species in an experimental setting (Ibrahim *et al*., 2019). Exogenous antioxidants such as ascorbic acid and vitamin E may be provided to infected rats and rabbits to reduce oxidative stress (Lokugamage *et al*., 2020). In trypanosome-infected animals, this vitamin therapy greatly decreased the degree and incidence of degeneration of tissues and organs, as well as parasitemia and anemia in some cases (Erol *et al*., 2019). *M. oleifera* is a highly nutritious plant that contains large amounts of vitamin B6, vitamin C, provitamin A in the form of beta-carotene, magnesium, and protein, among other nutrients. (Chaparro and Suchdev, 2000; Chhikara *et al*. 2020).

Moringa oleifera (*M. oleifera*) seed is being tested in a pilot study to see whether its nutrients and phytochemicals agents will help fight tissue damage caused by disease (Iliyasu *et al*., 2020a). Antibacterial activity and improved glucose tolerance in a rat model of diabetes, inhibition of Epstein-Barr virus activity in vitro, and reduction of skin papilloma in mice are just a few of the pharmacological properties of *M. oleifera* seeds (Vargas-Sánchez *et al*., 2019). *M. oleifera* Lam is one of fourteen species in the Moringaceae family. *M. oleifera* has anti-cancer, anti-inflammatory, hypoglycemic, and thyroid-status-regulating properties. The study aimed to see if graded doses of *M. oleifera* aqueous seed extract influenced testicular damage, gonadal and extragonadal sperm reserves, and testicular damage in Wistar rats infected with *T. brucei brucie*.

Materials and Methods

Twenty-five adult Wistar rats with an average body weight of 120 ± 0.7g were purchased from the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Maiduguri. The rats were acclimatized for 2 weeks in the Theriogenology Postgraduate Laboratory, University of Maiduguri Faculty of Veterinary Medicine. Thereafter, the twenty-five Wistar rats were randomly divided into five groups: A, B, C, D, and E, and each group has a total of five Wistar rats. The rats were fed with commercial pelleted poultry grower mash (Vital feed®) throughout the experiment, water was available at all times. The rats were housed in grated metal rat cages throughout the experimental period in the Theriogenology Postgraduate Laboratory, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Experimental Design

The rats were separated into groups A to E at random and inoculated with 1×10^9 virulent *T. brucei brucei* intra-peritoneal as describe by (Odeyemi *et al*., 2015) rats were allowed to stay one week post-infection to exhibit clinical signs before the commencement of the treatment with *M. oleifera* seed extract. The rats were treated daily around 10:00 AM for five weeks with 75 mg, 100 mg, 125 mg, and 150 mg of *M. oleifera* seed extract for groups A, B, C,

and D respectively. While group E was untreated control received 0.5 ml of water. Blood samples were collected every Monday between 10:00-11:00 AM for hematological indices as described by (Iliyasu *et al*., 20014; Opaluwa *et al*., 2015). All of the rats were humanely sacrificed at the end of the 6-week experiment, and their gonadal and extragonadal sperm reserves were assessed as described by (Iliyasu *et al*., 2014) testicular organs were harvested for the histopathology of the organs.

Plant Collection and Identification

The whole plant with fruits was collected during the dry season (November- March) at Anguwan Yusi, Sabon-gari Local Government Area of Kaduna State. The plant was authenticated and assigned with Voucher number 0571 by a taxonomist at the Herbarium Unit, Department of Biological Sciences, Faculty of Science, Ahmadu Bello University (ABU), Zaria, Nigeria. The Ahmadu Bello University Committee on Animal Use and Care gave clearance to all experimental protocols with clearance number ABUCAUC/2017/029.

Aqueous Extraction of *Moringa oleifera* Seed

The seeds *M. oleifera* were obtained from the fruits that dried under shade for 14 days to ease the shedding of the seeds. The dried seeds were made into powdered form (40 g) was weighed using a weighing balance and transferred into a one-liter beaker. Three hundred milliliters (300 ml) of distilled water were added to the powder and allowed to stand for 48 hours. Thereafter it was heated on a water bath at (60 °C) for 3 hours. Warm water was added continuously to the residue and subsequently filtered. The procedure was repeated three times at 10-15 minutes intervals and then the filtrate was evaporated to dryness on a water bath at (60 °C). The liquid extract was concentrated to dryness in vacuo at 40 °C using a rotary evaporator. The dried extract was kept at 4°C until it was required for use.

Acute Toxicity Study

The acute toxicity effects of an aqueous extract of *M. oleifera* seeds were investigated according to the method described by Lorke (1983). The experiment was done in two phases. In the phase I trial, nine rats were divided into three groups of three rats each at random. The first, second, and third groups were assigned and administered with *M. oleifera* aqueous seeds extracts at dose rates of 10, 100, and 1000 mg/kg, respectively, orally. In the second phase (II) of the trials, three rats were placed at random into three groups of one rat each. The groups were individually treated with three different doses of *M. oleifera* aqueous seeds extract based on the outcome of the initial trial. The median lethal dose (LD50) of *M. oleifera* aqueous seeds extract as an indication of its acute toxic effect was determined by taking the geometric mean of the highest dose that did not produce death and the lowest dose that produced death.

Source of Parasites

T. brucei brucei were sourced from the Department of Veterinary Parasitology and Entomology, University of Maiduguri, Nigeria. The parasites were injected into apparently healthy Wis-tar rats intraperitoneally at a dose rate of 10^9 cfu/mL and maintained in other rats by repeated passage according to the method described by Adeyemi *et al*. (2009). The parasitemia was monitored by preparing a thin film of blood smear obtained from the infected rat tail and viewed under light microscope ×100 magnification in agreement with the method described by Adeyemi *et al*. (2009).

Monitoring of Infected and Control Rats

The experimental rats were monitored regularly for the development of trypanosomosis clinical symptoms, such as morbidity and mortality. Every four days, parasitemia was first observed, and the degree of parasitemia was estimated by using rapid matching technique identified by (Herbert and Lumsden, 1976).

Detection of Parasitemia

Blood samples were obtained from the cardiac puncture of the rats to evaluate parasitemia following inoculation of the respective infected groups with *T. brucei brucei*. Wet mount and hematocrit buffy coat methods were used to test the blood samples, while the "rapid matching technique" was used to determine the degree of parasitemia. As described by Herbert and Lumsden, (1976) every four days of the experimental period.

Buffy Coat Examination

Blood was collected in two heparinized micro-hematocrit capillary tubes filled up to $3/4^{th}$ of their volume from each rat. One end of the tube was sealed

Table 1. Effects of aqueous seed extract of *M. oleifera* on Body weight, gonadal and extra gonadal dimension of Wistar rats experimentally infected with *T. brucei brucei*

Groups/Parameters	A	B	C	D	E
MOSE dose mg/kg	75	100	125	150	0.5 ml
Body weight (g)	130±1.2a	135±1.1	145±1.4b	153±1.3	160±1.6c
Testes weight (g)	2.2±1.2	2.1±1.1	2.3±1.4	2.3±1.3	2.2±1.6
Epididymis weight (g)	1.2±1.2	1.4±1.1	1.6±1.4b	1.5±1.3	1.3±1.6c

Values with different superscript within column are significantly different at $P<0.05$

with crystal sealant. The tube was sealed at the outer end and put in the microhematocrit centrifuge. The blood was centrifuged for 5 minutes at 10,000 g. To calculate the PCV, the tubes were removed from the hematocrit centrifuge and put on the microhematocrit reader. Anemic animals were described as those with a PCV value of less than 20 % as described by Murray *et al*. (1983). After reading the PCV, the capillary tubes were cut 1 mm below the buffy coat, including the top layer of the RBC, and the contents were expressed onto a slide and examined for trypanosomes using a dark ground-phase contrast microscope as described by Murray *et al*. (1983).

Wet Mount Examination

Wet blood films were prepared by placing a drop of blood on a clean, grease-free glass slide and placed a clean coverslip. The film was examined systematically for the presence of trypanosomes using × 40 magnification of the light microscope (Olympus, Japan).

Rapid Matching Technique for the Examination of *T. brucei brucei* Parasitaemia

Following Wet blood films preparation and examination, several parasites in each field under the microscope were matched with the standard reference pictures according to the method described by Herbert and Lumsden (1976). Each count per field was matched with logarithmic figures obtained from the reference tables. The logarithmic figures were converted to antilog and subsequently converted to absolute numbers, which reflected the number of trypanosomes per ml.

Histological preparations.

Rats in the control and treatment groups had their testes dissected. Before slide preparation, testicular organs were fixed in Bouin's fluid for 48 hours. They were again dehydrated in ethanol at different concentrations, cleared in xylene solution, and embedded in paraffin wax Luna (1960). The sections were then cut into 5 µm thick sections, placed on glass slides, and stained with hematoxylin and eosin (H&E) before being examined under light microscopy at ×40 magnifications. Gionee M2, Images plus 2.0 digital camera was used to capture photomicrographs (Motic China Group Ltd. 2014).

Statistical Analyses

Data collected for hematological analysis, gonadal and extragonadal sperm reserves were subjected to one-way ANOVA using GraphPad version 5.0 to determine significant difference $P<0.05$ among the groups.

Results

On days 7 and 10 after inoculation, the infected rats in groups A and B showed a loss of appetite and marked weakness. The rats in groups C and D were dull and sluggish compared to the rats in Group E. (control group). The infected groups continued with some discharges from the eyes up to day 17 post-inoculation when one of the infected rats in group A died. There was a progressive decreased in body weight and some of the reproductive organs of the infected groups compared to the control group as presented in (Table 1). There was a progressive increase in gonadal and extragonadal sperm reserves of the infected treated groups compared to the control group as presented in (Table 2). Similarly, the

mean PCV, WBC, WBC differential and RBC counts, were decreased significantly in group A later progressively increased in groups C and D compared to the control group as presented in (Table 3).

Table 2. Effects of aqueous seed extract of *M. oleifera* on gonadal and extragonadal sperm reserves of Wistar rats experimentally infected with *T. brucei brucei*

Groups/Parameters	A	B	C	D	E
MOSE dose mg/kg	75	100	125	150	0.5 ml
Gonadal sperm reserves (sperm× 106)	213±1.1a	221±2.1b	250±0.0c	259±2.6d	295±0.6e
Extra gonadal sperm reserves(sperm× 106)	115±1.1	160±2.1b	153±0.0c	167±2.6d	120±0.6e
Right epididymis (sperm× 106)	60.0±1.1a	90.2±2.1b	96.5±0.0c	98.7±2.6d	69.4±0.6e
Left epididymis (sperm× 106)	55.0±1.1a	69.8±2.1	56.5±0.0c	68.3±2.6d	50.6±0.6e

Values with different superscript within column are significantly different at $P<0.05$
Key: MOSE= *M. oleifera* seed extract

Table 3. Effects of aqueous seed extract of *M. oleifera* of haematological parameters of Wistar rats experimentally infected with *T. brucei brucei*

Groups/Parameters	A	B	C	D	E
MOSE dose mg/kg	75	100	125	150	0.5 ml
PCV (%)	22.9 ± 2.8^{a}	25.2 ± 2.17^{b}	38.1 ± 1.5^{c}	40.7 ± 8.1^{d}	47.0 ± 2.2^{e}
WBC ($\times10^{3}/\mu L$)	20.7±2.8	16.3 ± 2.1^{b}	16.5±1.5	15.5±1.1	9.7 ± 0.2^{e}
Lymphocytes	64.8 ± 2.8^{a}	71.8 ± 2.1^{b}	61.7±1.5	51.6 ± 5.5^{d}	42.5 ± 0.2^{e}
Monocytes	81.8±2.8	52.1 ± 2.1^{b}	53.6±1.5	60.7±5.8	47.0 ± 0.1^{e}
Neutrophils	82.8±2.8	74.0 ± 2.1^{b}	72.3±1.5	59.3±1.2	30.0 ± 0.3^{e}
Eosinophils	4.0 ± 2.8^{a}	0.2 ± 2.1^{b}	0.1 ± 0.0^{c}	0 ± 0^{d}	17.0 ± 0.1^{e}
Basophils	0.8 ± 1.1^{a}	1.7±1.9	1.7±1.5	1.6 ± 1.2^{d}	10.0 ± 0.1^{e}
RBC ($\times10^{6}/\mu L$)	5.1 ± 2.8^{a}	5.8±1.3	6.5±0.8	7.8±1.3	8.5±0.4e

Values with different superscript within column are significantly different at $P<0.05$
Key: PCV=packed cell volume; WBC= white blood cell; RBC= red blood cell; MOSE= *M. oleifera* seed extract

Histology of the testes

Rats in the control group showed normal seminiferous tubules and interstices in their testes. (Figure 1). The morphology of the testes of rats treated with *M. oleifera* aqueous seed extract showed a significant reduction in the activity of spermatogenic cells in the seminiferous tubules, as well as hyperemia and fluid exudation into the interstices.

In all of the groups infected with *T. brucei brucei*, testicular pathology was found, and the severity of the pathology was dose-dependent. The germinal epithelium of the seminiferous tubules of rats given a high dose of *M. oleifera* aqueous seed extract was found to be involved in spermatogenesis, and the interstices were improved. (Figure 2).

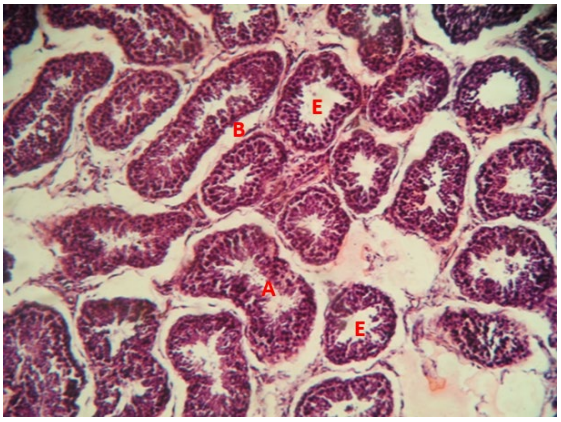

Figure 1. Microphotography of Testis of control group (E) rat shows seminiferous tubules (A) with normal spermatogenic cells and interstitial spaces (B) containing Leydig cells.

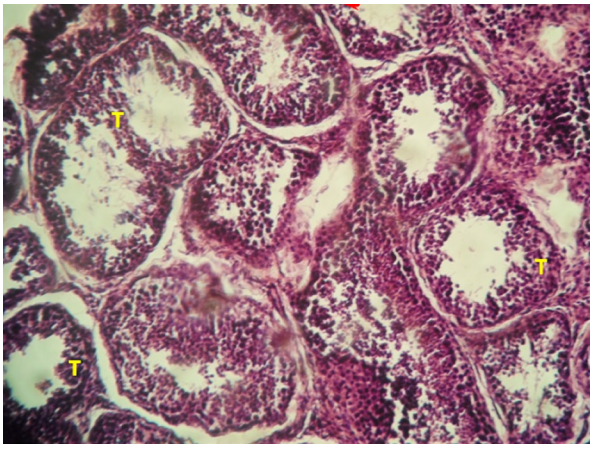

Figure 2. Microphotography of testis of the group (A) rats treated with aqueous seed extract of *M. oleifera* at a dose rate of 75 mg/kg (T) showing spermatogonia with poor seminiferous tubules activities.

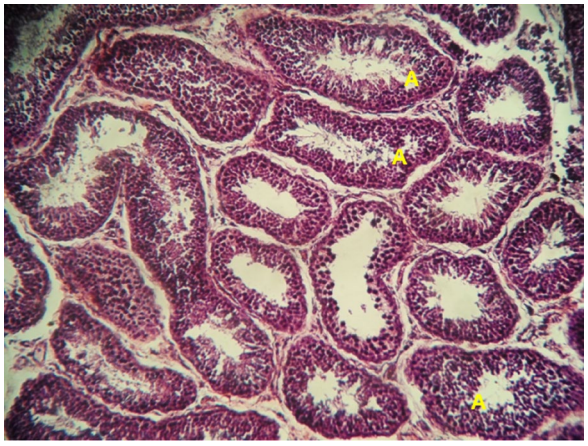

Figure 3. Microphotography of testis of the group (D) rats treated with aqueous seed extract of *M. oleifera* at a dose rate of 150 mg/kg (A) showing spermatogonia with effective seminiferous tubules activities.

Similarly, spermatogonia and seminiferous tubules activities were efficient as shown in (Figure 3).

Discussion

The dimension of anemia is a reliable indicator of disease status and efficient output in trypanosome infected animals (Stijlemans *et al*., 2018; Abdullahi *et al*., 2018; Shousha *et al*., 2020). According to Joachim *et al*. (2019), trypanosome infection causes anemia in animals as a result of massive erythrophagocytosis by the host's enlarged and active mononuclear phagocytic system (MPS). In the current study the low PCV showed in the treated groups may be attributed to acute hemolysis due to the parasites' rapid growth. The hematological findings are consistent with those of previous research (Odeniran *et al*., 2018; Chaparro and Suchdev, 2019; Goel and Maurya, 2020). The RBC concentration increases in a dose dependant fashion following administration of the *M. oleifera* aqueous seeds extract. The increase in RBC concentration could be to the antioxidant properties of the extract and this is consistent the findings of a previous research which showed that trypanosome infection increases the vulnerability of red blood cell membranes to oxidative damage, likely as a result of glutathione depletion or reduction on the surface of red blood cell RBCs as reported by Revin *et al*. (2019) and Narayan *et al*. (2019). It was also reported by Raftery *et al*. (2020) that the degree and duration of parasitemia, and perhaps even the disease status, are generally a reflection of severity of anemia which is in consonant with the findings of the present study where the rats exhibited a protracted parasitemia. The presence of *T. brucei brucei*, in the current study might by responsible for the decrease in the mean values of PCV, RB, and Hb estimations. Al-Otaibi *et al*. (2019) and Shahrajabian *et al*. (2019) reported that the presence of anemia is similarly indicated by the decline in these hematological parameters which is also proven by the findings of the current study.

In the present study, the infected rats showed signs of fatigue and loss of appetite after being inoculated. The rats were dull and uninterested in comparison to the control group and there was an increase in the white blood cell (WBC) count in the infected rats which signified sign of infection, it might be attributable to the augmented role of antioxidant and immune stimulant activities of *M. oleifera* aqueous extract which aided the optimal body immune response mechanism to combat invading parasites by increasing the release of more WBC into circulation which is in accord with the findings of Shittu *et al*. (2018).

In the present study, there was an increase in gonadal and extragonadal sperm reserves which could stimulate sexual drive of the treated rats and this might also be attributable to the nutritional value and

antioxidant properties of *M. oleifera* aqueous seeds extract as reported by (Sekoni, 1994) and Iliyasu *et al*. (2020) which was also in lined with the report of Abou-Elkhair *et al*. (2020) in respect to the traditional herbalists findings in Asia and Africa that used the seed of *M. oleifera* extract to treat sexual inadequacy, stimulate sexual vigor, and treat trypanosomiasis in livestock, without regard for the scientific validity of the claims. Clinical toxicity symptoms such as respiratory distress, salivation, anemia, extreme weight loss, and hair change were not observed in all groups, although one death was recorded in group A during the second week of the trial, which could be attributed to a decline in immune status which was similar to the finding of Shahrajabian *et al*. (2019).

In addition, the *T. brucei brucei* infection damaged the morphology of the testes, which was characterized by a decrease in spermatogenic activity, oligospermia in the lumen of the seminiferous tubules, and the presence of interstitial exudates is also similar to the findings of Mutwedu *et al*. (2019). These findings are also in line with a previous study reported by Obidike *et al*. (2007) and Mohamed *et al*. (2019). In the present study, it was observed that *M. oleifera* seed extract at a dose rate of 150 mg/kg could enhance the gonadal sperm reserve characteristics and cause substantial amelioration of *T. brucei brucei* infection. This effect was also observed in group given 100 mg/kg of *M. oleifera* aqueous seeds extract as against 75 mg/kg of *M. oleifera* aqueous seeds extract in Wistar rats.

Conclusion

The current findings showed that ingestion of *M. oleifera* aqueous seed extract improves reproductive success in adult male rats and mitigates the effects of *T. brucei brucei* on rat testes. It also supports traditional healers' claims about the use of *M. oleifera* seeds as a semen quality enhancer or medicine. As a result, this study has shown that restoring the spermatogenic activities of the testes during trypanosomasis is successful and healthy, as well as serving as an alternative treatment for trypanosomiasis in rats.

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Conflict of Interest

The authors declared no conflicts of interest exist.

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