

Research Article:

Comparative Anti-malarial and Toxicological Properties of the Stem Bark Extracts of *Nauclea latifolia* and *Terminalia glaucescens* Against *Plasmodium berghei*-infected Mice



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ABSTRACT

Background: Anti-plasmodial activities of the methanol stem bark extracts of *Nauclea latifolia*, and *Terminalia glaucescens* were investigated in mice infected with *Plasmodium berghei*.

Methods: A total of 24 *Plasmodium berghei* infected (*P. berghei* infected) mice were divided into 8 groups of 3 each. Groups A-F were given 100, 300 or 500 mg/kg of either extracts. Groups G and H received 2 mL normal saline (negative control) and 5 mg/kg of chloroquine (positive control), respectively. The drugs and extracts were administered orally once daily for five days.

Results: Alkaloids and flavonoids were the most and abundant metabolites in the extracts, respectively. The extract of *Nauclea latifolia* (*N. latifolia*) and *Terminalia glaucescens* (*T. glaucescens*) had Median Lethal Dose LD₅₀ of >5000 mg/kg and 3808 mg/kg, respectively. In vivo anti-plasmodial studies revealed that the highest suppression (66.79% and 65.37%) and mean survival days (27.67±1.45 and 30.33±0.33) were recorded for the groups treated with 500 mg/kg *N. latifolia* or *T. glaucescens*, respectively. The infected but untreated groups survived only for 9.33±0.88 days while chloroquine treated groups lived for 31.33±0.88 days. The body weight and Packed Cell Volume (PCV) of rats treated with 500 mg/kg, *N. latifolia* or *T. glaucescens* significantly increased (P<0.05) compared to those in the infected but untreated groups. There was a significant loss (P<0.05) in body weight and PCV of the mice treated with 100 mg/kg of *T. glaucescens* compared to those in other treated groups.

Conclusion: The extracts exhibited anti-plasmodial activities in mice, therefore, they may be considered potential candidates for new anti-malarial agents.

Keywords: Phytochemicals; Toxicity; Anti-malarial drugs; *Nauclea latifolia*; *Terminalia glaucescens*

Introduction

Malaria is one of the deadly infectious diseases globally and remains one of the oldest life-threatening parasitic diseases in Africa, despite the control programs [1]. Malaria affects the lives of almost all people but young children and pregnant

women are at the highest risk where morbidity and mortality occur the most [2]. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. It is estimated that about 80% of rural populations still depend on herbal medicine and validations of these claims in treating malaria have yielded significant benefits [3].

The use of plants in traditional medicine in the treatment of various diseases is known since the beginning of human history [4]. Traditional folk medicine derived from plants has always guided researchers to search for novel drugs aimed at improving the health of humans and animals. The active ingredients extracted from these plants have led to the development of several lifesaving drugs, which are being used today in various communities globally [1].

Nauclea latifolia (*N. latifolia*), also known as *Sarcocephalus latifolus* (*S. latifolus*) is commonly called the African pincushion tree. It is used in traditional medicine in various regions of West Africa in the treatment of illnesses, such as malaria, pain and many infections [5]. The plant is known for its anti-inflammatory, anti-pyretic and anti-plasmodial properties, among others [6].

Terminalia glaucescens (*T. glaucescens*) belongs to the Combretaceae family, believed to have anti-malarial activity but little systematic research has been conducted to explore its properties. The plant has been used traditionally to treat pain, dysentery, venereal diseases, coughs, dysmenorrhea, intestinal parasitosis, ulcers, leprosy and malaria [7]. The ethanolic extract has exhibited aldose inhibition plus anti-plasmodial and anti-cytotoxic effects [8]. In this study, the phytochemical and anti-plasmodial activities of *T. glaucescens* and *N. latifolia* were investigated in mice infected with *Plasmodium berghei* (*P. berghei*).

Materials and Methods

Collection and identification of plant materials: The stem bark of *N. latifolia* and *T. glaucescens* were collected with the help of local traditional medicine practitioners in Nso, North west region of Cameroon. Their scientific identification and authentication were established at the National Herbarium in Yaoundé, Cameroon (voucher specimen No.: 56386/HNC and 33322SRF/Cam). The plant samples were dried at room temperature and transferred to the laboratory in the Department of Biochemistry at Federal University of Technology, Minna, Nigeria, for further analyses.

Animals: Adult, Swiss albino mice, weighing 18-25 g, were obtained from the National Veterinary Research Institute (NVRI) in Vom, Plateau State, Nigeria. The animals had access to standard mice feed and water ad libitum. The mice were separated into male and female groups three weeks before starting the study.

Parasite: The parasite, chloroquine sensitive *P. berghei* (NK-65), was obtained from the National Institute of Pharmaceutical Research National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria. Parasitized erythrocytes were obtained from an infected donor mouse via cardiac puncture and were maintained by continuous reinfection in mice (serial passages).

Preparation of the plant materials: The plant materials were dried at room temperature and homogenized into a fine powder, using an electric blender. A 100 g sample of each plant material was extracted with 750 mL of 80% V/W methanol at 60°C for 2hr in a reflux extractor. The extract was filtered with a muslin cloth followed by Whatman paper filter No.1. The filtrate was evaporated to dryness using a water bath (45°C). The extracts were stored in tightly stoppered bottles and kept in a refrigerator at -4°C until used for further assays.

Quantitative phytochemical analyses: The plant extracts were subjected to quantitative phytochemical analyses, using standard procedures. Total phenols concentration was estimated using an established method [9]. The total alkaloids were measured according to the method of Harborne [10], saponins were determined as described by Oloyede [11] while tannins and flavonoids were assayed as described by Association of Official Analytical Chemists [12].

Acute toxicity of the extracts: The acute oral toxicity studies of the extracts were estimated in mice, using the modified Lorke's method [13, 14]. The study was conducted in 2 phases with the extract concentrations at 10, 100 or 1000 mg/kg in phase 1, and 1600, 2900 and 5000 mg/kg in phase 2. The LD₅₀ was calculated as the squared root of the product of the lowest lethal dose and the highest non-lethal dose. Specifically, this was the geometric mean of the consecutive dose for which 0 or 100% survival rates were recorded in the second phase. The median oral lethal dose was determined, using the following formula: LD₅₀ = Maximum survival dose x minimum lethal dose.

Evaluation of anti-plasmodial activity: Rane's test was performed as reported by Iyiola et al. [15] with minor modifications. On the first-day, adult mice were inoculated intraperitoneally by injection of 0.2 mL of standard inoculum containing about 1.0×10⁷ erythrocytes parasitized with *P. berghei*. These mice were randomly divided into 8 groups (A to H) of 3 animals each. The administration of the extracts and standard drug (chloroquine) was initiated three days' post infection when par-

asitaemia had been established. The drugs and extracts were administered orally once a day at 24-hour intervals for five days (D0 to D4).

Mice in groups A, B and C were given of *N. latifolia* at graded doses of 100, 300 or 500 mg/kg, respectively. Groups D, E and F were given *T. glaucescens* extract at 100, 300 or 500 mg/kg, respectively. Group G mice received only 2 mL normal saline (negative control) while group H received 5 mg/kg chloroquine (positive control). Parasitaemia were counted on a daily basis according to the method described by Jigam et al. [16]. The body weights and Packed Cell Volume (PCV) were monitored during the study period. The percent suppression of parasitaemia (Equation 1) and the mean survival days (Equation 2) of the mice were calculated for each test concentration, using the following equations:

$$1. \quad \% \text{ Suppression} = \frac{\text{Parasitaemia in (-ve) control} - \text{Parasitaemia in test group}}{\text{Parasitaemia in (-ve) control}} \times 100$$

2.

$$\text{Mean Survival Time (MST)} = \frac{\text{Sum of survival days of all mice in a group}}{\text{Total number of mice in that group}}$$

Results

Percent yield and phytochemical composition: Stem barks of *N. latifolia* and *T. glaucescens* had the extract yields of 17.32% and 18.71%, respectively. The quantitative phytochemical constituents of the crude extracts are presented in Table 1. The alkaloids were the most and while flavonoids were the least abundant secondary metabolites from both plants. Phenols and saponins were present at high concentration in *T. glaucescens* while flavonoids were found at high concentration in *N. latifolia*.

Acute oral toxicity profile of the extracts: The extracts of *N. latifolia* showed no signs of toxicity at any dose as shown in Table 2. The animals were calm and

Table 1. Phytochemical constituents of the extracts of *N. latifolia* and *T. glaucescens* stem barks

Phytochemicals	mg / 100g	
	<i>N. Latifolia</i>	<i>T. Glaucescens</i>
Total phenols	0.71±0.02 ^c	0.86 ±0.00 ^c
Flavonoids	0.11±0.01 ^a	0.08 ±0.05 ^a
Tannins	0.53±0.09 ^b	0.55 ±0.08 ^b
Alkaloids	1.35±0.03 ^d	1.39 ±0.05 ^d
Saponins	0.49±0.00 ^b	1.20 ±0.01 ^d

Values are the Mean±SEM (n=3)

Values in a column with different superscript alphabet are significantly different (P<0.05);

The increasing order of significance are a<b<c<d

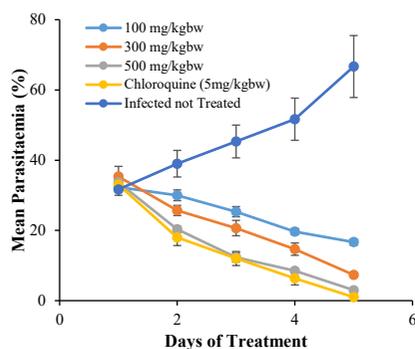


Figure 1. The course of parasitaemia in infected mice treated with *N. Latifolia* extract

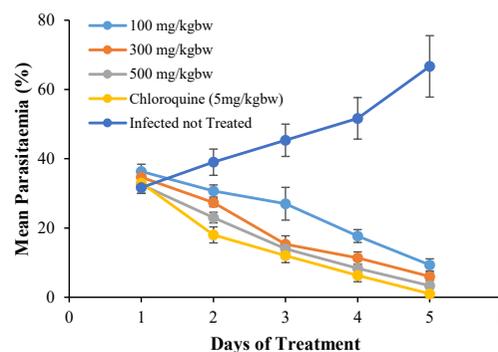


Figure 2. The course of parasitaemia in infected mice treated with *T. glaucescens* extract

Table 2. Oral acute toxicity of the stem bark extract of *N. latifolia* in mice

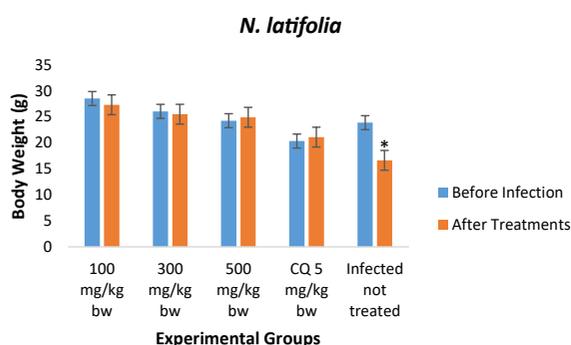
Dose (mg/kg)	Observations	Mortality/Survival
10	Animals appeared normal without any physical changes or deaths in 72 h.	0/3
100	Animals were normal with appropriate feed uptake and other physical activities.	0/3
1000	The animals were generally devoid of any impairment, exhibiting normal physical appearance. There were no deaths recorded in 72 h.	0/3
1600	Normal activities were observed for all the animals in the dose group. Absence of mortality at 72 h.	0/3
2900	Animals demonstrated slight irritability, poor feed uptake with erythema of the extremities but absence of death at 72 h.	0/3
5000	Animals demonstrated reduced feed uptake and physical activities with depressed respiration. However, none of the rodents died at 72 h.	0/3

Table 3. Acute oral toxicity effect of the stem bark extract of *T. glaucescens* in mice

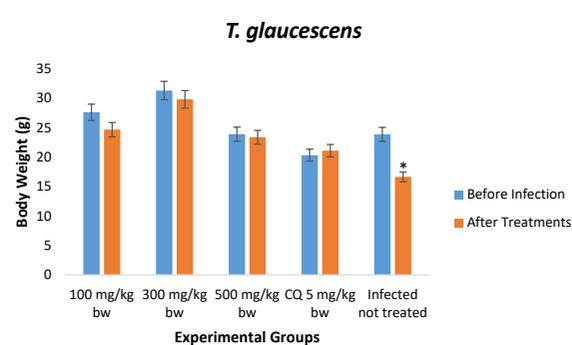
Dose (mg/kg)	Observations	Mortality/Survival
10	Animals appeared normal without any physical changes or deaths in 72 h.	0/3
100	Animals were normal with appropriate feed uptake and other physical activities.	0/3
1000	Animals were generally devoid of any impairment, exhibiting normal physical appearance. There were no deaths recorded in 72 h.	0/3
1600	The animals were generally devoid of any impairment, exhibiting normal physical appearance. There were no deaths recorded in 72 h.	0/3
2900	Animals demonstrated slight irritability, poor feed uptake with erythema of the extremities but one rat died at 72 h.	0/3
5000	Animals demonstrated reduced feed uptake and physical activities with depressed respiration. One rat died before 72 h.	1/3

showed no strange reactions on the first phase of the experiment. However, as the dosage increased in the second phase, the animals were restless but the initial signs of weakness were reversed on the same day. All of the animals survived the experiments throughout the study. The LD₅₀ was estimated to be greater than 5000 mg/kg.

The toxicity studies of *T. glaucescens* in the first phase did not cause any animal death (Table 3). The mice appeared normal, and no treatment-related signs of toxicity were observed during and after the treatments. In the second phase, as the dosage increased (1600 to 5000 mg/kg), the extract produced such physical signs as gasping for air, depression, and low food intake. Also, one death occurred after

**Figure 3.** Effect of *N. latifolia* extract on bodyweight changes in *P. berghei* infected mice

CQ: Chloroquine; *: Significant difference before infection and after treatment ($P < 0.05$)

**Figure 4.** The effect of the *T. glaucescens* extract on body weight in *P. berghei* infected mice

CQ: Chloroquine; *: Significant difference before infection and after treatment ($P < 0.05$)

Table 4. Parasite (%) and (MST) on *P. berghei*-infected mice treated with the stem bark extracts of *N. latifolia* and *T. glaucescens*; (n=3)

Treatment (mg/kg)	Suppression (%)		MST (Days)	
			Mean±SEM	
	<i>N. latifolia</i>	<i>T. glaucescens</i>	<i>N. latifolia</i>	<i>T. glaucescens</i>
Infected but not treated	0	0	9.33±0.88 ^a	9.33±0.88 ^a
100	31.73	48.37	15.67±1.45 ^b	21.67±1.20 ^b
300	55.76	59.61	25.00±2.65 ^c	28.00±1.53 ^c
500	66.79	65.37	27.67±1.45 ^c	30.33±0.33 ^c
CQ 5	69.99	69.99	31.33 ±0.88 ^d	31.33±0.88 ^c

Different superscripts in the columns are significantly different at P<0.05

CQ: Chloroquine

Values followed by different superscript alphabets are significantly different (P<0.05).

The increasing order of significance are a<b<c<d.

the administration of *T. glaucescens* at 2900 mg/kg followed by another death at 5000 mg/kg. Thus, the LD₅₀ was determined to be 3808 mg/kg.

Anti-plasmodial activities of the extracts: The administration of the extracts was started 72 h post infection. On day one, the parasitaemia was determined before treatment in all the animals to be within 30%-37% and kept declining post treatment through day five. As shown in Figure 1 and 2, after five days of treatment, the parasitaemia was significantly lower (P<0.05) in the groups treated with the extract compared to the infected but untreated mice (negative control). The parasitaemia declined to below 15% in all groups treated with the extract or chloroquine (<2%; positive controls). The groups treated with 500 mg/kg, exhibited the lowest parasitaemia as compared with other doses, thus indicating

a dose-dependent effect. However, parasitaemia was significantly lower (P<0.05) in the group treated with chloroquine compared to the highest dose of the extract (500 mg/kg) in all of the treated groups. The parasitaemia increased to 66.67% in the infected but untreated group (negative controls) after five days of being infected.

Effects of the extracts on parasitaemia suppression: The parasitaemia suppression and mean survival times of the mice treated with the extracts of *N. latifolia* and *T. glaucescens* are presented in Table 4. The highest suppression (66.79%) and mean survival days (27.67±1.45) was found in the groups treated with the *N. latifolia* extract at 500 mg/kg. The infected but untreated group survived only 9.33±0.88 days while chloroquine-treated groups lived for 31.33±0.88 days. For *T. glaucescens*, the highest suppression (65.37%) and mean survival

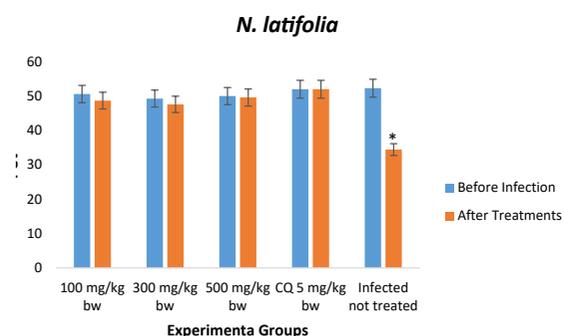


Figure 5. Effect of *N. latifolia* extract on packed cell volume (PCV) in *P. berghei* infected mice

CQ: Chloroquine; *: Significant difference before infection and after treatment (P<0.05)

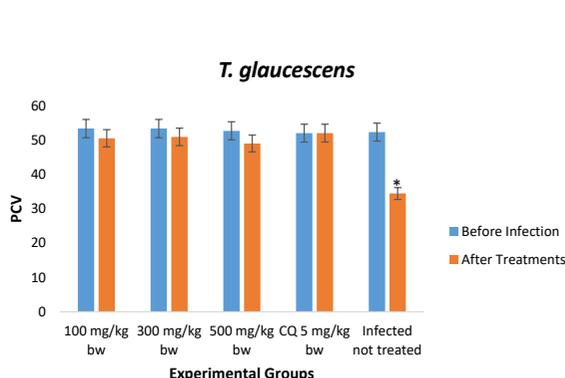


Figure 6. Effect of *T. glaucescens* extract on the pack cell volume (PCV) in *P. berghei* infected mice

days (30.33 ± 0.33) was recorded for the groups treated with the extract at 500 mg/kg.

Effects of the extracts on changes in body weight: There was no significant difference ($P > 0.05$) in the animals' body weights before and after treatment (Figure 3). The mice treated with *N. latifolia* and *T. glaucescens* exhibited a significant increase ($P < 0.05$) in weight compared to the infected but untreated group (negative controls). The chloroquine group (positive controls) showed a significant increase ($P < 0.05$) in weight compared to those treated with the extracts. There was a significant loss ($P < 0.05$) in the body weight of mice treated with 100 mg/kg of *T. glaucescens* compared to other treated groups (Figure 4).

Effect of the extracts on packed cell volume: The results indicated that the PCV for all of the treatments were not significantly different before the infection and after the treatment (Figures 5 & 6). The mean PCV recorded 5 days after treatment with the extracts showed an insignificant decrease in all of the treatment groups. However, The PCV of the infected but untreated group showed a significant decrease ($P < 0.05$) in PCV compared to those in the treated groups.

Discussion

The preliminary phytochemical constituents detected were phenols, alkaloids, saponins, tannins and flavonoids (Table 1). Phytochemical constituents have been frequently reported for their therapeutic benefits in herbal medicines. The secondary metabolites, such as alkaloids have shown significant anti-malarial activities by blocking the protein synthesis in *Plasmodium falciparum* [17]. Saponins, flavonoids and tannins have been suggested to act as primary anti-oxidants or free radical scavengers, inhibiting the oxidative damages caused by malaria [18].

These secondary metabolites could have elicited the observed anti-plasmodial activity either singly or synergistically with each other, through the inhibition of protein synthesis and preventing the invasion of new red blood cells by the plasmodium parasites. The concept of phytochemical constituents contributing to anti-malarial activity is consistent with the results reported by Jigam et al. [16]. These authors investigated the anti-plasmodial, analgesic and anti-inflammatory effects of the extract of *Guira senegalensi* (*Combretaceae*) leaves in mice infected with *P. berghei*. Ettebong et al. have also reported

the association of the phytochemicals with anti-malarial activity of *Eleucine indica* [19].

Safety margin of the extracts: The LD_{50} estimated to be greater than 5000 mg/kg for *N. latifolia* is consistent with the findings of Ogugua et al. who also reported the potential protective effects of the methanolic extract of *N. latifolia* on the liver and kidneys in experimental animals [20]. On the other hand, the LD_{50} of 3808 mg/kg for *T. glaucescens* is not consistent with those found by Konan et al. who suggested the toxicity of the aqueous *T. glaucescens* extract being greater than 5000 mg/kg [21]. This discrepancy could be attributed to the differences in the polarity of the extraction solvents. However, the highest dose (500 mg/kg) of the extracts, used to treat the infected mice with anti-malarial activity, was far lower than 1600 mg/kg, indicative of the extract safety. Any orally administered test substance with lethal dose less than 1000 mg/kg or higher than three times the minimum effective dose can be considered nontoxic [22].

Effect on physical activity: There was a general increase in the physical activities of the mice treated with the extracts compared with that in the parasitized but untreated mice. The results showed that the physical activities of the extracts-treated mice were better than that observed in the untreated animals. This could be due to the ameliorating effect of the plant extracts on malaria infection as confirmed by the reduction in parasite multiplication of the treated rats. The anti-malaria activity of *T. laucescens*, and *N. latifolia*, as demonstrated in this study, justifies the traditional use of these plants for the treatment of malaria in Nso, Cameroon.

Dose and time dependence: The extracts also showed that their anti-plasmodial activity in mice infected with *P. berghei* was exposure duration-dependent and required significantly higher amounts of the extract compared with the standard drug (chloroquine), as evidenced by the percentage of inhibition of the parasite and improving the survival time. The extracts may be considered as active anti-malarial agents, based on their ability to produce a minimum inhibition of parasitaemia (30%) and offering a greater percent of survival time, compared to the results noted in the infected but untreated mice [23].

Moreover, the extracts administered at a daily dose of 500 mg/kg for five days resulted in >50% reduction of the parasitaemia. This performance is likely to be improved if the extracts are purified and the active molecules identified. The observed activity suggests

that the extracts may suppress the parasitic condition to lower levels in the long-run and its application in traditional medicine may be further justified. Our findings are also supported by previous studies that discovered anti-plasmodial potentials in the methanol extracts of *Eucalyptus camadulensis* and *N. latifolia* leaves in laboratory animals [24-26].

Although there are ample research published on some members of *Combretaceae* plant family for their anti-plasmodial and anti-protozoal activities [27]; however, studies on *T. glaucescens* are very scarce. To the best of our knowledge, the present study is the first in vivo research on the anti-plasmodial activity of the methanol extracts of *T. glaucescens*.

Weight loss: The weight loss is a notable manifestation in mice infected with *P. berghei*. This is likely to be due to the parasite depressant action on the appetite and other disturbed metabolic functions [28]. The body weight can be used to evaluate the anti-malarial activity of the mice treated with the above extracts [29]. The extracts showed a dose-dependent, preventive effect on body weight loss as compared to that observed in the untreated animals. However, the weight loss could be attributed to anti-nutritive factors that may be present in the crude extracts [30].

For instance, tannins inhibit growth by decreasing the digestion coefficient of most nutrients and the coagulation of proteins. The increase in body weight at higher doses of the crude extract may be due to the fact that higher level of parasitaemia significantly lowers the initial body weight so the significant change might be due to the regain of initial weight loss by the curative effects of higher doses of the extracts. The implication is that lower doses lack enough concentration of the active ingredients. Similar suggestions have been made by Jigam et al. who studied the *Guiera senegalensis* leaf extracts [16].

Packed Cell Volume: Erythrocyte fragility, reduced PCV and life-threatening anemia is commonly observed in mice infected with *P. berghei* [30]. The effect of PCV was reversed after five days of treatment with the extracts, though there were insignificant differences in the absolute values of PCV documented among the groups in our study. Further, there was no significant difference among the PCV values before and after infection. The extracts were able to reverse the decline in PCV because of their effect on the parasite. However; the PCV in the infected but untreated mice (negative controls) was much lower than those of the treated groups.

Rodent malaria causes a decline in PCV, occurring progressively 48 hours after the infection onward with no amelioration expected. The untreated mice suffered because of erythrocyte destruction, either by parasite multiplication or by destruction or entrapment in the spleen. This occurs due to the presence of many abnormal erythrocytes stimulating phagocytosis in the spleen [31]. These findings are similar to the work of Kabiru et al. who studied the anti-plasmodial effect of the methanolic extracts of *Eucalyptus camadulensis* leaves [24]. These authors reported similar findings for reduced *Bush mango* in the infected but untreated mice.

Conclusions

The methanolic stem bark extracts of *N. latifolia*, and *T. glaucescens* exhibited comparable and significant activities against *P. berghei* infection in mice. Thus they could be considered as potential candidates for the development of new anti-malarial agent for clinical experiment and eventual use in humans, especially in the practice of traditional medicine.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Committee on Ethics for Medical and Scientific Research at Federal University of Technology, Minna, Nigeria. We duly observed the principles governing the use of experimental animals, set out by the university, based on the internationally accepted principles for animal use and care, as contained in the Canadian Council on Animal Care Guidelines and the review protocol.

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Author's contributions

Design: Eustace Bonghan Berinyuy and Emmanuel Olofu Ogbadoyi; Conduction of the lab work: Emmanuel Olofu Ogbadoyi; Supervision: Adamu Yusuf Kabiru; Participated in the study design and co-supervision: Mann Abdullahi.

Conflict of interest

The authors declare no conflict of interests in conducting this study.

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