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Preliminary Study: Neurobehavioural Effects of *Nauclea latifolia* and *Emilia sonchifolia* in Mice Infected with *Plasmodium berghei berghei*

Innocent A. Edagha[1*], Koofreh G. Davies[2], Sunday O. Ita[2], Aquaisua N. Aquaisua[1] and Blessing E. Anwana[2]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, P.M.B 1017, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Physiology, Faculty of Basic Medical Sciences, University of Uyo, P.M.B 1017, Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was designed to examine the effects of ethanolic leaf extracts of *Nauclea latifolia* and *Emilia sonchifolia* on anxiety, fear and locomotion in mice infected with *plasmodium berghei berghei*. Thirty male Swiss albino mice weighing between 26-30g divided into five groups with six mice in each group. Group 1 served as the Control group and was treated with 0.2ml of normal saline, Group 2 served as the parasitized non-treated, Group 3, was parasitized and treated with Coartem®, Group 4 was parasitized then treated with *Emilia sonchifolia*, Group 5 was parasitized and treated with *Nauclea latifolia* and Group 6 was parasitized and treated with a combination of *Nauclea latifolia* and *Emilia sonchifolia* respectively. The mice were passaged with the parasite intraperitoneally and then administered extract orally using an orogavage cannula for a duration of 5 days. Behavioural tests were performed pretreatment (day 6 after parasite passage) and post-treatment (day 11). The results obtained showed that grooming frequency and stretch attend

**Corresponding author: Email: innocentedagha@uniuyo.edu.ng, iedagha@yahoo.com;*

frequency were significantly ($p<0.001$) lower in groups 3-5 compared with the Control group. The combined extract treatment in group 5 was significantly ($p<0.001$) reduced compared with the parasitized non treated group. Line crossing duration was significantly ($p<0.001$) lower in groups 2 and 4 but significantly higher in groups 3 and 5 compared with the control group. This preliminary study consolidates the view of herbal practitioners that the extract is effective in reducing anxiety and fear and enhances increases locomotion in plasmodium berghei infected mice.

Keywords: Malaria; fear and anxiety; Locomotion; Nauclea latifolia; Emilia sonchifolia; Plasmodium berghei; mice.

1. INTRODUCTION

Polyherbal preparations as decoctions or concoctions have continued to enjoy wide acceptance by a great number of people in both the rural and urban areas of Nigeria as an alternative malaria therapy, despite the availability of some affordable conventional antimalarial drugs. The extraction of bioactive agents from plants is one of the most intensive areas of natural product research today, yet the field is far from being exhausted [1]. Researchers with interest in natural products have intensified their efforts towards scientific evaluation of traditional medicines [2]. Several articles have reported on the use of *Nauclea latifolia* and *Emilia sonchifolia* for their antiplasmodial and anxiolytic activities amongst many other ethnobotanical properties.

Antidepressant/myorelaxant and anti-anxiety activities of *Nauclea latifolia* have been reported by [3]; and antiplasmodial activities has been reported by [4-9]. Anti- inflammatory and analgesic has been reported by [10] in the leaves (aqueous extract); wound healing activity has been reported by [11] in stem bark (methanolic extract) and antimicrobial activities by [12,13]; [14] in the stem bark (chloroform extract), and [15] leaves (aqueous and chloroform).

Emilia sonchifolia possess antioxidant activities [16-19]; analgesic and anti-inflammatory activities [20,21]; anticancer activities [22-24]; anti-cataract activities [25-28]; antimicrobial activity [29]; anti-diabetic [30]; anticonvulsant activity [31]; anti-fever activities [32,33].

This study aims to determine the effects of ethanolic leaf extract of *Nauclea latifolia* and *Emilia sonchifolia* on locomotion, fear and anxiety in mice infected with *plasmodium berghei,* to ascertain the possible side effects of this intervention in the neurobehaviour of animals undergoing antimalaria treatment.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty male Swiss Webster mice where obtained from the animal holding facility of Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria and acclimatized for two weeks before the start of the experiment. They were allowed access to water and feed *ad libitum*.

2.2 Plant Collection

Fresh leaves of *Nauclea latifolia* and *Emilia sonchifolia* was obtained at the medicinal farm of the Department of Pharmacology and Toxicology, University of Uyo. They were identified and authenticated by the Curator; at the Herbarium of the Department of Pharmacognosy and Natural medicine with voucher numbers UUH/67 (g) for *Nauclea latifolia* and UUH/10(e) for *Emilia sonchifolia* deposited.

2.2.1 Ethical approval

All procedures involving animals in this study conformed to the guiding principles in the care and use of animals [34] and the Faculty of Basic Sciences, University of Uyo code of ethics for the use of laboratory animals.

2.3 Plant Extraction

The extraction was done on fresh leaves as 1100g of *Nauclea latifolia* and 700g of *Emilia sonchifolia*, macerated in 96% ethanol (Sigma Aldrich, Germany) in a flat bottom flask and were kept for 72 hours at room temperature. The macerated leaves were then filtered and the filtrate concentrated in water-bath at 45oC to dryness with *Nauclea latifolia* yielding 85.41g while *Emilia sonchifolia* yielded 15.71g.

2.4 Phytochemical Screening

Qualitative assay for the presence of phytochemical constituents was carried out using the standard procedures by [35] to reveal the presence of constituents such as alkaloids, saponins, flavonoids, tannins, cardiac glycosides and anthraquinones.

2.5 Parasite Inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 ml of infected blood containing about 1×10^7 *Plasmodium berghei* parasitized erythrocytes. The inoculums consisted of 5×10^7 *Plasmodium berghei* erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations [36].

2.6 Dosage

All extracts dosage was determined after toxicity test (LD50) was done using the modified Lorke's method [37]. Only the 10% of the LD50 of the extracts was administered as shown in Table 1 below.

Table 1. Experimental design

Treatment groups & dosage (n=5)	Duration
Control (0.3ml) normal saline	11 days
PB non-treated	11 days
PB + Coartem® 5mg/kg	6+5 days
PB + ES325mg/kg	6+5 days
PB + NL500mg/kg	6+5 days
PB + ES325mg/kg + NL500mg/kg	6+5 days

NL – Nauclea latifolia, ES – Emilia sonchifolia, PB – Plasmodium berghei

2.7 Open Field Maze

The Open field maze is built of plywood and measures 72 by 72cm with 36cm walls. One of the walls is made of clear Plexiglas so that the mice can be observed from the front of the apparatus as well as from the top which also without a cover. Lines painted blue divide the floor of the open field into forty-nine 5 x 5 cm squares, and these lines are used to assess locomotor activity. The centre square (15 x 15 cm) is formed from the four inner squares and this square is highlighted with a black marker. A sheet of clear Plexiglas covers the floor. All animal testing was conducted under diffuse lighting conditions via a 60-Watt white light bulb. It provides simultaneous measures of locomotion, anxiety and fear [38]. The behavioural test was done before the start of treatment (day 6, after the passage of mice) and at the end of treatment (day 11).

2.7.1 Procedure

Mice were carried to the test room in their home cages and tested one at a time. The mice were scooped up in a small plastic container from their home cages and placed randomly into one of the four corners of the open field. They were allowed to explore the apparatus for 5 minutes, while taking scores of their behaviours. After the 5 minutes test, the mice were scooped up from the open field with the plastic container and returned to their home cages. The open field was cleaned with 70% ethyl alcohol and permitted to dry between trials. The behaviours scored included: 1. Number of line crossing; frequency with which the mice crossed one of the grid lines with all four paws. 2. Centre square entries; frequency with which the mice crossed one of the red lines with all four paws into the central square. 3. Duration of stay in the central square. 4. Rearing frequency and duration.

2.8 Elevated Plus-Maze (EPM)

The Elevated Plus-Maze was built according to the description of Lister [39]. The apparatus is in the configuration of a + (that is, shape of a cross) and comprised two open arms (25 x 5 x 0.5 cm) across from each other and perpendicular to two closed arms (25 x 5 x 16 cm) with a centre platform (5 x 5 x 0.5cm). The open arms had a very small (0.5 cm) wall to decrease the number of falls, whereas the closed arms had a high (16 cm) wall to enclose the arm [40]. The entire apparatus was 50 cm above the floor. The apparatus was made of white transparent Plexiglas materials.

2.8.1 Procedure

Mice were carried into the test room in their home cages and were handled by the base of their tails at all times. Mice were placed in the central square of the Plus-Maze facing an open arm and were then allowed to explore the apparatus for 5 minutes. The maze was then

cleaned with a solution of 70% ethyl alcohol and allowed to dry between tests. Behaviours scored were: 1. Open Arm Entries: Frequency with which the animal entered the Open arms. All four of the mouse's paws should be in the open arms to be regarded as an entry. 2. Open Arm Duration: Length of time the animal spent in the open arms. 3. Head Dipping: Frequency with which the animal lowered its head over the sides of the open arms towards the floor. 5. Rearing: Frequency with which the animal stands on its hind legs or leans against wall of the maze with front paws.

2.9 Statistical Analysis

The data obtained from the study were expressed as mean value ± standard error of mean (SEM). The data was statistically analyzed using one way ANOVA and the difference between the means of groups were considered significant at ($p<0.05$) confidence level (Primer of Biostatistics: The Program © McGraw-Hill version 3.01) while the Histogram and error bars was prepared with MS Office Excel 2007.

3. RESULTS AND DISCUSSION

This study was designed to assess the effect of oral administration of ethanolic extracts of *Nauclea latifolia* and *Emilia sonchifolia* in parasitized mice during locomotion, anxiety and fear tests using the Open field maze (OPM) and the Elevated plus maze (EPM). Behaviour such as line crosses and rearing are used as a measure of locomotion and exploratory activity. A high frequency of these parameters indicates increased locomotion and exploration. The frequency of line crosses measures the horizontal locomotor behaviour and represents the horizontal covered [41]. Line crossing is the frequency with which the mice cross each of the lines with all four paws. In Figs. 1 and 2, the final line crossing and central square entry was significantly ($p<0.5$) increased in groups 3,4,5, and 5 compared to the control, unlike at their initial measurements, indicative that antiplasmodial properties of Coartem®, and the extract, ameliorated the adverse effect of the parasite in the infected mice, thereby improving to some degree their motor activity, but the final line crossing and central square entry in Group 2 was significantly decreased possibly due to the burden of the infection. Fig. 3 shows that *P. berghei* only in group 2, had a significantly ($p<0.05$) lower central square duration compared with the control. Damage in the primary motor area, without involvement of adjacent cortex or underlying white matter, is seldom encountered clinically [42]. *Plasmodium* iRBC, platelets, and immune cells have been suggested to accumulate in the central nervous system during ECM [43,44]. A destructive lesion in area 4 results in paresis (weakness) of the opposite side of the body. The muscles involved are flaccid if the damage is restricted to the precentral gyrus.

In Fig. 4, the initial and final rearing frequency was significantly decreased compared to the control. Rearing measures exploratory behaviour or otherwise vertical locomotor activity. When rearing, the animals stands upright on the hind-legs the often using the tail as support while visually exploring the environment [45]. Rearing allows the mice to explore its horizon obtaining information that requires it taking an action as to what and represent exploration. Comparatively, the treated groups experienced more horizontal locomotion than vertical locomotion. Table 2 of the EPM assessed the open and closed arm durations as well as head dipping, as assessments for fear and anxiety. Group 2 showed significantly ($p<0.05$) higher final closed arm duration compared to the control, which may be due to the generalized muscle fatigue as well as reduced health status of the non-treated mice. The final Stretch attend postures (SAP) in the OPM test showed that Group 2 and 3 were significantly ($p<0.05$) increased compared to Groups 1,4,5 and 6 likewise in Table 3 of the EPM, also shows that Group 3 was significantly ($p<0.05$) increased compared to Groups 5 and 6. SAP is the frequency with which the animal demonstrated forward elongation of head and shoulder followed by retraction to its original position. These are risk assessment behaviors of fear and anxiety which indicates that the animal is hesitant to move from its present position of comfort to a new position. Thus a low level of this posture indicates a low level of anxiety and fear [46].

Stress and feeling of anxiety are among the most universal emotions. Stress is defined as the set of all organic reactions to physical, psychic, infectious or other aggressions, capable of disturbing homeostasis [47]. Anxiety test is based on the contrasting tendency of mice to explore a novel environment against the aversive properties of an open, brightly lit or elevated space [48]. In addition, in the open field, anxiety behavior may be triggered by two factors namely, individual testing (the animals being separated from its social group) and agoraphobia (as the

area is very large relative to the animal). In Fig. 5, the initial freezing duration (FD) was significantly ($p<0.05$) increased in Groups 4, 5 and 6 compared to the control, but was subsequently decreased in the final measurement except Group 2 which had a prolonged FD possibly due to the level of infection of the *Plasmodium berghei*. However, both the initial and final grooming frequency (GF) was significantly decreased in the treated groups compared to the control indicative that perhaps anxiolytic properties from the extracts may have played a role. Grooming is a displacement response and is associated with anxiety in animals when they are introduced into a novel environment [49]. Grooming duration is the time the animal spent licking or scratching itself while stationary. It is possible that the anxiolytic properties from the ethanolic extract enhance the action of the inhibitory neurotransmitter GABA by binding to a subtype of its postsynaptic receptor that occurs abundantly on the surfaces of neurons in the amygdale and other parts of the limbic system [42]. Fig 6 shows result for urination and defecation which are known indicators of fear and anxiety. Groups 2 and 3 had significantly ($p<0.05$) increased defecation post-infection, whereas groups 4 and 6 had significantly ($p<0.05$) reduced urination. This may suggest that bioactive chemicals in the extract down-regulated receptors and connectivity in the amygdala, a key center of fear.

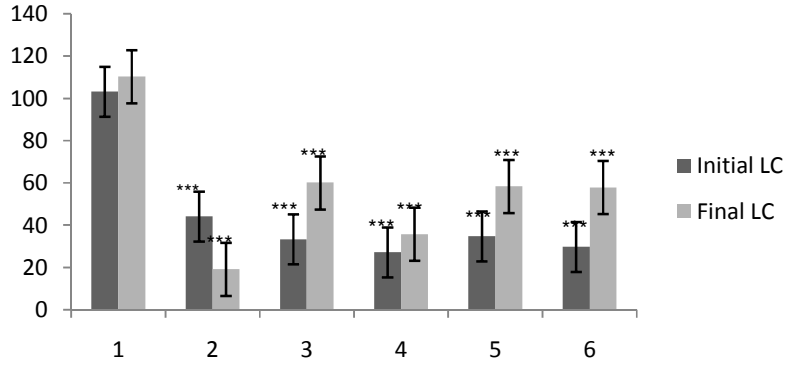

Fig. 1. Comparison of the frequency of line crosses (Initial and Final) in the open field maze in mice before and after treatments between treated groups (2-6) and control group (1)

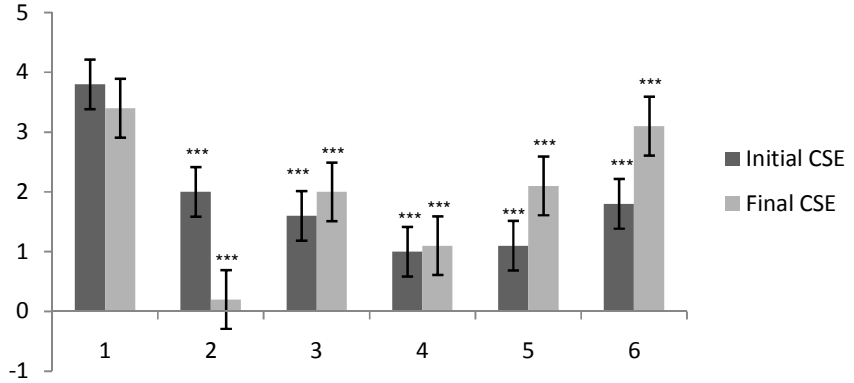

Fig. 2. Comparison of the frequency of centre square entries (Initial and Final) in the open field maze

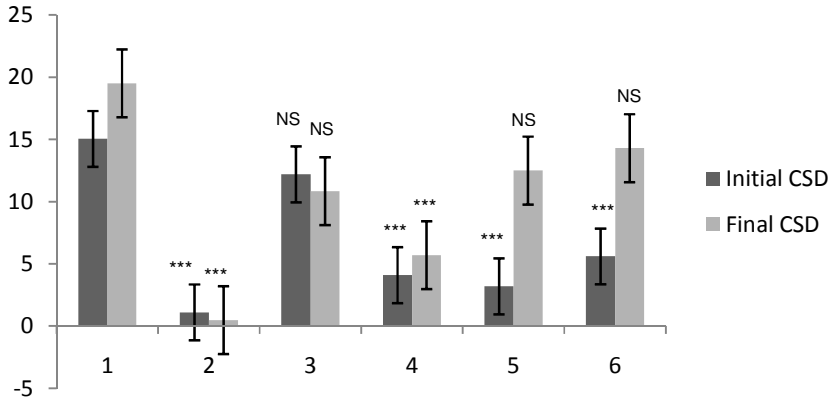

Fig. 3. Comparison of the frequency of centre square duration (Initial and Final) in the open field maze

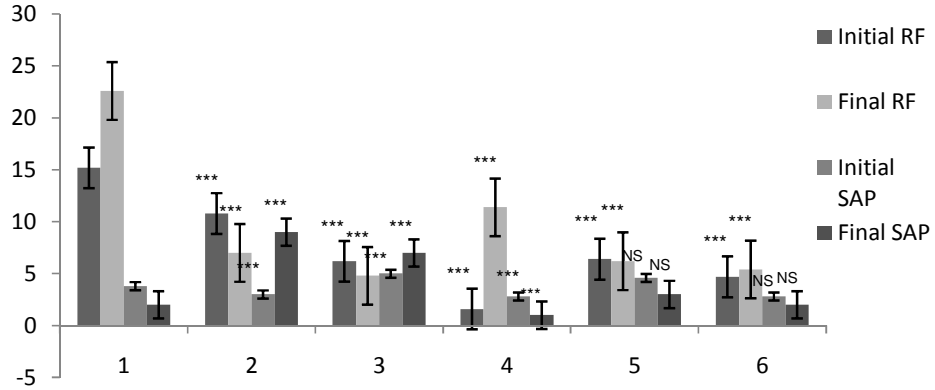

Fig. 4. Comparison of the frequency of rearing (RF) and Stretch Attend Posture (SAP) (Initial and final) in the open field maze

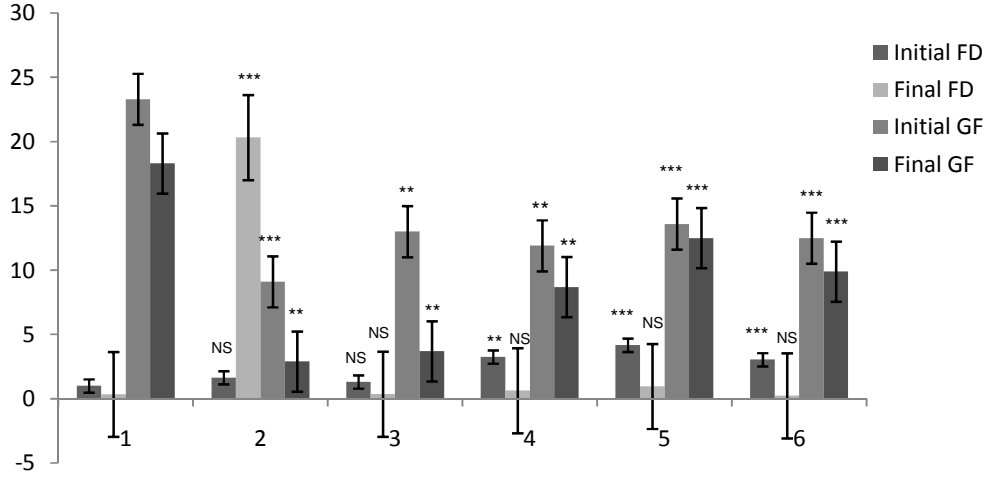

Fig. 5. Comparison of the initial and final of Freezing Duration (FD) and Grooming Frequency (GF) in the open field maze

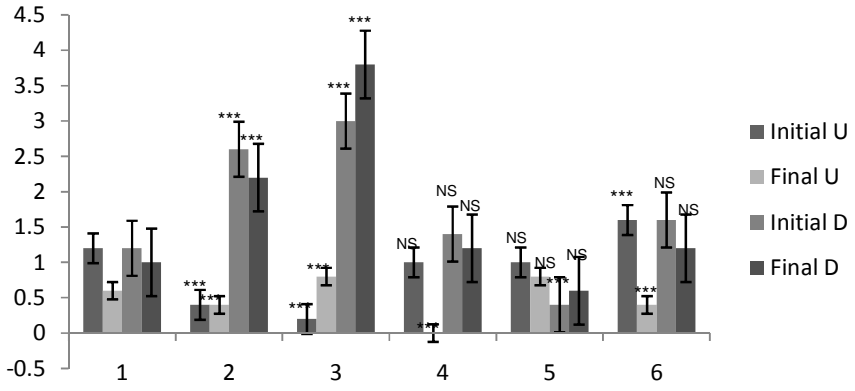

Fig. 6. Comparison of the frequency of Urination (U) and Defecation (D) (Initial and Final) in the open field maze

Table 2. Comparison of open, closed arm durations and head dipping in elevated plus maze

Treatment (n =5)	Open arm duration (secs)		Closed arm duration (secs)		Head dipping/5min	
	Initial	Final	Initial	Final	Initial	Final
Control	28.36±2.84	29.44±1.07	21.79±6.01	18.85±3.53	9.00±1.41	3.20±0.86
PB	1.21±0.36***	39.58±10.66	16.94±7.17^{NS}	39.54±5.03**	4.00±1.30***	2.40±0.51
PB+Coartem	4.85±2.68***	9.22±2.60^{b}	5.91±2.69 ^{NS}	29.80±4.20	1.00±0.55***	0.40±0.24
PB+ES	5.87±3.23***	39.21±6.14	24.58±7.37^{NS}	24.29±1.65	10.20±1.02^{d}	0.80±0.37
PB+NL	28.48±7.98^{a}	33.74±9.48	18.90±5.82^{NS}	16.91±2.99^{c}	7.00±1.26^{e}	4.20±1.16
PB + ES+NL	30.96±8.23 ^{a}	26.85±4.40	25.51±7.17^{NS}	18.90±2.96^{c}	9.40±0.68^{d}	7.60±2.11**^{,f}

*Data are mean ± SEM values. *P<0.05; **P<0.001; ***P<0.0001 and NS – Not significant compared with control.*
a – Groups 5 and 6 statistically significant at P<0.0001 compared with groups 2, 3 and 4; b – Group 3 statistically significant at P<0.05 compared with groups 2 and 4; c – Group 5 and 6 statistically significant at P<0.001 compared with group 2; d – Groups 4 and 6 statistically significant at P<0.0001 compared with groups 2 and 3
e – Group 5 statistically significant at P<0.0001 compared with group 3; f – Group 6 statistically significant at P<0.001compared groups 1, 2, 3, 4, and 5

Table 3. Comparison of stretch attend posture, urination and defecation in elevated plus maze

Treatment (n=5)	Stretch attend posture		Rearing freq./5min		Urination		Defecation	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	2.80±0.73	3.80±0.73	7.60±1.96	2.20±0.49	0.40±0.24	0.00±0.00	0.00±0.00	0.40±0.24
PB	5.20±1.20^{a}	4.60±0.87	1.80±0.58***	3.40±0.60^{d}	0.60±0.40^{NS}	1.00±0.32*^{,e}	0.40±0.24	1.00±0.45
PB+Coartem	0.40±0.24^{b}	7.40±2.27^{c}	1.00±0.32***	0.60±0.40	0.40±0.24^{NS}	1.00±0.55*^{,e}	1.00±0.55	0.40±0.24
PB+ES	4.20±0.73	2.20±0.44	1.60±0.93***	1.80±0.80	0.40±0.24^{NS}	0.40±0.24	2.20±0.73*^{,f}	1.40±0.24^{g}
PB+NL	4.00±1.18	1.60±0.40	1.00±0.55***	0.60±0.24	0.00±0.00^{NS}	0.00±0.00	1.40±0.24	0.00±0.00
PB + ES+NL	1.00±0.32	0.40±0.24	2.20±0.66***	1.20±0.37	0.40±0.24^{NS}	0.00±0.00	1.00±0.34	0.60±0.24

*Data are mean ± SEM values. *P<0.05; **P<0.001; ***P<0.0001 and NS – Not significant compared to control*
a – Group 2 statistically significant at P<0.05 compared to group 6; b – Group 3 statistically significant at P<0.05 compared to groups 2, 4 and 5
c – Group 3 statistically significant at P<0.05 compared to group 5 and 6; d – Group 2 statistically significant at P<0.05 compared to groups 3, 5 and 6; e – Groups 2 and 3 statistically significant at P<0.05 compared to groups 1, 5 and 6; f – Group 4 statistically significant at P<0.05 compared with group 2; g – Group 4 statistically significant at P<0.05compared with group 5

5. CONCLUSION

From this study, it was observed that *Nauclea latifolia* and *Emilia sonchifolia* is able to decrease fear and anxiety in parasitized mice while increasing their locomotion and exploratory activity. This may be indicative of its use as antimalarial treatment, and possible psycho-active ingredients are present which can be isolated for the management of neurological conditions such as epilepsy and convulsion. Further work is required to establish the depth of this possibility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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