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Research Article

Involvement of Acetylcholinesterase and Nitric Oxide on the Behavioural Changes Induced by *Harugana madagascariensis* Extract in Mice Infected with and without *Plasmodium berghei* Malaria Parasite

Njan A. A[1], Iwalewa E. O[2*], Akinpelu L. A[2], Ilesanmi O. R[2], Obuotor E.M[3], Olawuni I. J[3] and Olorundare O. E[1]

Department of Pharmacology and Therapeutics1, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin. Department of Pharmacology2, Faculty of Pharmacy, and Department of Biochemistry3, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

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Abstract

We evaluated the role of acetylcholinesterase (AChE) and nitric oxide (NO) in the behavioural effects of *Harugana madagascariensis* extract in mice, infected with and without *Plasmodium berghei* in novelty–induced behavioural models. This is also to examine the possible mechanism through which the plant reverses the behavioural effects during malaria infection. The novelty –induced rearing (NIR), grooming (NIG), locomotion (LC) and exploratory (EXP) behaviours were observed in non-infected and malaria infected mice using the suppressive; the curative and the prophylaxis modes of malaria treatment(s). In independent experiments, the ability of the Harungana stem bark extract treatments in changing the activity of AChE and NO in the brain cells of infected mice were evaluated. The result showed that the extract decreased the various behaviours in both infected and non-infected mice. The reserved observable novelty-induced behaviours in curative test were not significant ($p > 0.05$), but were significant in suppressive test. Likewise, the prophylactic treatment showed a significant ($p < 0.05$) reversal and increase in behavioural patterns after extract administration in infected mice. It is likely that the reduction in AChE and increase in NO activities by the extract contributed significantly to the behavioural effects exhibited. The significant reversal from depressive state in the prophylactic treatment especially could suggest the mechanism through which the constituents of this plant are best employed in traditional medicine for malaria. It is also possible that NO production and reduction in AChE activities are important for the behavioural changes.

Keywords: *Acetylcholinesterase (AChE), nitric oxide (NO) inhibition, rearing, grooming, locomotion, exploratory behaviours, Harugana madagascariensis, Plasmodium berghei*

INTRODUCTION

Parasites and diseases are recognized as agents of behavioral changes as they influence the mood, behaviours of their hosts. Parasitic infections can have significant behavioral effects even when parasites load is of sub-clinical value (Kavaliers et al., 1999). However, until recently little attention has been paid to the neuromodulatory substrates that mediate these behavioral changes. In some of our recent studies, ethnopharmacology approach was used to study the behaviour with pharmacological tools of neuromodulatory mechanisms (Akomolafe et al., 2006; Ayoka et al., 2006; Adeyemi et al., 2007). As such, this approach is appropriate for, and has been applied to, the analysis of the effects of endoparasites (e.g. protozoa) on a number of behaviours (e.g. pain inhibition, learning and memory, responses to predators and anxiety, mate selection) in selected host–parasite systems (Kavaliers et al., 1999 and 2000). Ethnopharmacology approach could suggest a promising direction by which neuromodulatory mechanism that underlies the effects of parasites on behavior can be explained. It is revealed that during the course of infection such as malaria in humans and animals, there is alteration in the functions of the body systems which may result in inactiveness, depression, changes in moods and behaviours, pains or abnormal temperature (Bowman and Rand 1980). Remedies from medicinal plants have been used from time immemorial to restore the body functions back to its normal state but some common compounds found in plants have been shown to be toxic (Willcox and Bodeker 2004). This, coupled with the emergence of resistance to the existing agents against malaria parasites have made the quest for newer antimalarial drugs from medicinal plants a continuous effort.

Harungana madagascariensis (Lam.ex.Poiret Hypericaceae) leaves and stem bark extracts are used in ethnomedicine for malaria and anaemia. Studies have shown that H. madagascariensis stem bark has therapeutic efficacy in malaria, diabetic and exhibited anti-anaemic properties (EMEA, 1999; Tona et al., 2000; Erah et al., 2003; Kamanzi Atindehou et al., 2004). The work of Rukunga and Simons, (2006), and Ndjakou Lenta et al., (2007) showed the anti-plasmodial effects of six isolated compounds from the root bark of *Harungana madagascariensis* and other natural products in vitro. Recently Umukoro et al., (2013a, 2013b and 2013c) revealed the different pharmacological effects of

**Author for correspondence:* +234-7083519364
E-mail: eoiwalewa@yahoo.com

Jobelyn® an herbal formulation that contains *Harungana madagascariensis* in the treatment of convulsion, psychosis, depression and aggressive behavioural activities in mice. Thus the specific objective of the study is to assess the effect of acute treatment of the extract on novelty induced behaviour (rearing and grooming), locomotive and exploratory activities (as in open field and head dip tests) in mice infected with and without rodent malaria parasite using three modes of malaria treatments (suppressive, curative and prophylaxis).

MATERIALS AND METHODS

Plant materials and preparation of the extracts:
Harungana madagascariensis samples (stem bark) were collected in the main campus of Obafemi Awolowo University, Ile-Ife. The plant was identified by Mr. O. A. Oladele of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife and a voucher specimen with number FHI 107392 was kept at the herbarium of the Forestry Research Institute of Nigeria, Ibadan. The ethanolic extract was made by soaking 325g of the powdered stem bark in 70% ethanol for 24 hours. After filtration, the extract was evaporated in vacuo in a rotary evaporator to dryness, yielding 9.0 g of the brown crude extract.

Experimental animals
Swiss albino mice (18 – 22g) of both sexes were bred and maintained in the animal house of the department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife. The mice were housed under natural lighting conditions and maintained at room temperature. They were fed with commercial marsh diet (Guinea Feeds brand, Bendel Feeds Nigeria) and given water ad libitum. The "principle of laboratory animal care" (National Institute of Health –NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985).

Parasites:
The NK 65 chloroquine sensitive strain of Plasmodium berghei was donated generously by the Malaria Research and Reference Reagent Resource Center (MR4) Manassas, VA, USA, and was obtained from Institute of Medical Research and Training courtesy of Prof. O. G. Ademowo.

Drugs, Chemicals and Equipment
Plexiglass cage measuring (45cm x 25 cm x 25 cm); Hole wooden board with 16 evenly spaced holes. Ethanol (BDH, Chemical Limited, London), Chloroquine (May & Baker), and Pyrimethamine (Wellcome).

In vivo antimalarial model
In the in vivo model, albino mice (Swiss strain) 6 to 7 weeks old weighing 18 – 22gm were infected with rodent malaria parasite Plasmodium berghei from a donor mouse. The in vivo model in mice was carried out as follows: (a) the suppressive test (early infection) using 4- days suppressive test to assess blood schizonticidal action (Peters and Robinson 1992); (b) the curative test (established infection) using modified Rane test method (Ryley and Peters 1970), (c) the resipository test (as a causal prophylaxis) (Peters 1965).

Behavioural studies in suppressive test
Twenty five albino mice were chosen and randomly divided into 5 groups, each group containing 5 mice. A donor mouse infected with parasites (parasitaemia of about 17–30%) was anesthesized with chloroform and blood collected through cardiac puncture with a sterile and non- pyrogenic disposable needle syringe. The blood was diluted with normal saline such that 0.2ml of blood contained approximately 1 X 107 infected red blood cells. The inoculum of 0.2ml is given to each of the 25 mice intraperitoneally (i.p). The test compound, *H. madagascariensis* stem bark extract at dose levels of 20, 40, 80 mg/kg were administered once daily for 4 days, starting from day 0 (D0) and continued on D1, D2 and D3 by the subcutaneous route. A parallel test was run using chloroquine (CQ, 5 mg/kg) in group 4 as a standard drug. The fifth group serves as a control infected group and receive no treatment. Each mouse in different treatment and control groups was observed for novelty induced behaviour- rearing, grooming, locomotion for 30 minutes after the subcutaneous administration of the extract on day 1 and day 4 in suppressive test. Each mouse was subsequently observed for exploratory behaviour for 5 minutes.

Behavioural studies in curative test
Twenty five albino mice were chosen and randomly divided into 5 groups, each group containing 5 mice and were infected with parasites (parasitaemia of about 17 – 30 %). The animals were left for 7 days for the infection to be established. Different dose levels of *H. madagascariensis* at 20, 40, 80 mg /kg were administered subcutaneously once daily for 5 days from D7 to D11. A parallel test was run using chloroquine (5mg/kg) in group 4 as a standard drug. The fifth group serves as a control infected group and receive no treatment. Each mouse in different treatment and control groups was observed for novelty induced behaviour- rearing, grooming, locomotion for 30 minutes after the subcutaneous administration of the extract on day 7 and day 11 for curative test. Each mouse was subsequently observed for exploratory behaviour for 5 minutes.

Behavioural studies in prophylaxis test
Another twenty five albino mice were also chosen and randomly divided into 5 groups, each group containing 5 mice. *H. madagascariensis* extract at dosage of 20, 40, 80 mg /kg, pyrimethamine (1.2 mg /kg) in group 4 as a standard drug were administered subcutaneously once daily for 3 days. The fifth group serves as a control infected group and receive no treatment. A donor mouse infected with parasites (parasitaemia of about 17 – 30 %) was used to inoculate the mice. Each mouse in different treatment and control groups (control infected and pyrimethamine group) was observed for novelty induced behaviour- rearing, grooming, locomotion for 30 minutes after the intraperitoneal administration of the parasites on day 0 and day 3 for prophylactic test. Each mouse was subsequently observed for exploratory behaviour for 5 minutes. All the parameters measured were recorded.
Determination of NO release and Assay for NO / NO2 nitrite concentration

NO production was determined by measuring the accumulation of nitrite in the supernatant of the brain tissue homogenate using the Griess reagent. Inhibitory effect of extract on NO production by brain cells were evaluated using a modified method from that previously reported (Banskota et

al., 2003). Nitrite in brain cells were measured, as previously described by Green et al., (1982), by adding 1ml of clear supernatant from the homogenate to 1.5 ml Griess reagent (1% sulfanilamide and 0.1% N-w1- naphthyl-ethylenediamine in 5% phosphoric acid), for 20 min at room temperature. The Absorbance was measured 530 nm using a Spectramax 250 spectrophotometer. The nitrite concentration in μM was calculated from sodium nitrite standard curve.

Acetylcholine esterase (AChE) inhibition assay

AChE enzyme inhibition was evaluated using the Ellman's procedures as modified by Perry et al., (2000). Spectrophotometric method was used to determining the specific activity of extract and CQ. The assay method employs the substrate acetylthiocholine iodide (ATChI), which is hydrolysed to acetate and thiocholine. The thiocholine subsequently reacts with dithiobisnitrobenzoate (DTNB) to produce a yellow colour monitored by colorimeter at 412 nm. The assay was carried out by adding 2.0 ml of phosphate buffer (100 mM, pH 8.0) to 100 μl of the test sample and 100 μl of 10 mM DTNB. The mixture was vortexed and allowed to equilibrate at room temperature for 5 min. The reaction was initiated by the addition of 100 μl of freshly prepared ATChI. The change in absorbance at 412 nm was measured at 30 sec interval for 4 min. An enzyme blank (control) was also run through the same procedure except that the buffer was used instead of the substrate. Assay was carried out in duplicate. Specific activity (μmoles/min/ml) was calculated as total activity/protein concentration (mg/ml)

Statistical analysis:

The results were expressed as mean + S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA followed by Benferroni t-test against a single control, $p < 0.05$ were considered significant

RESULTS

Effect of *H. madagascariensis* extract on novelty-induced behaviours in non-infected mice examined indicated a significant dose dependent reduction in activity (Table 1). However, CQ increases these behaviours when compared to the control. Table 2 showed a general depression of the extract, but not with CQ in mice infected with malaria during suppressive test. The changes were significant ($p < 0.05$) in all behaviours when in D1 was compared to D4.

Table 1:
Effects of Harungana madagascariensis stem bark extract and chloroquine (CQ) on novelty-induced and exploratory behaviours In non-infected mice. The data represent mean + SEM of at least 5 animals.

Treatment (mg / kg)	Rearing/30 min	Grooming/30 min	LC/30 min	Exploratory/5 min
Normal saline (0.3 ml)	121 ± 14.2	44.6±4.7	137 ± 11.1	38.7 ± 2.8
Extract (20)	43.0 ± 17.8*	31.0 ± 11.6	87 ± 29.2	40.6 ± 3.9
(40)	16.6 ± 9.5*	17.8 ± 7.8 *	88.8 ± 16.1	34.9 ± 3.8
(80)	19.7 ± 2.4*	10.2 ± 1.0 *	59.6 ± 7.5*	26.0 ± 1.8*
CQ (10)	112.6 ± 3.71	73.4 ± 11*	194 ± 9.9*	48.8 ± 4.57

The extract showed significance effect in all the behavioural components of rearing, grooming, locomotion and exploratory at 80 mg/kg of the extract when compared to normal saline. The extract at 20 mg/kg was only significant in rearing compared to other behaviours. Chloroquine showed significance only in grooming and locomotion when compared to normal saline

Table 2:
The suppressive test effect of *Harungana madagascariensis* stem bark extract on novelty-induced and exploratory behaviours in infected mice on D1 and D4. The data represent mean ± SEM of at least 5 animals. *Significance difference was observed in locomotion and exploratory behaviours using student's t-test at ($p<0.05$).

		Day 1		
Treatment (mg / kg)	Rearing/30 min	Grooming/30 min	Locomotion/30 min	Exploratory/5min
CNI	121 ± 14.2	44.6±4.7	137 ± 11.1	38.7 ± 2.8
CI	64 ± 18.59	51.2 ± 7.17	72.2 ± 6.15	40.2 ± 5.41
20	84.8 ± 7.32	126 ± 18.21(**)	75.6 ± 2.4	24.4 ± 1.69
40	40 ± 6.86	70 ± 22.51	60.2 ± 14.40	30.6 ± 3.14
80	30.6 ± 4.33	67.4 ± 8.43	64.4 ± 6.61	28.4 ± 3.97
CQ	71.6 ± 10.46	73.6 ± 8.39	66 ± 3.15	45 ± 5.32
		DAY 4		
	Rearing/30min	Grooming/30min	Locomotion/30min	Exploratory/5min
CI	47.6 ± 17.85	64 ± 22.8	39 ± 5.02*	21 ± 2.41*
20	38.8 ± 10.12*	74.8 ± 11.85*	22 ± 3.73*	20 ± 1.70
40	30.8 ± 11.40	36.8 ± 3.50*	21 ± 1.76*	20 ± 2.63*
80	21.2 ± 1.59	38.8 ± 4.16*	35.8 ± 5.46*	21 ± 1.70*
CQ	152.8 ± 24.7* (**)	86.8 ± 22.84	62.8 ± 8.65(**)	35.8 ± 1.93(**)

No significance difference was observed on day 1 when all the treated groups were compared to control infected (CI) at ($p> 0.05$) using student's t-test. But when subjected to ANOVA followed by Benferroni t-test (represented by **) against control infected (CI), significance was observed in grooming at 20mg/kg of the extract.

Table 3:
The curative test effect of *Harungana madagascariensis* stem bark extract on novelty-induced and exploratory behaviours in infected mice on D7 and D11. The data represent mean ± SEM of at least 5 animals. *Significance difference was observed in behaviours using student's t-test at ($p<0.05$).

		DAY 7		
Treatment (mg / kg)	Rearing/30 min	Grooming/30 min	Locomotion/30 min	Exploratory/5 min
CI	128 ± 31.06	41.2 ± 4.66	199.2 ± 43.50	37.4 ± 6.26
20	48.4 ± 12.9	53.4 ± 14.78	88.2 ± 20.31	24 ± 4.32
40	52.8 ± 27.68	71.4 ± 11.16	93 ± 41.34	38.4 ±11.76
80	64.8 ± 23.86	76.6 ± 17.52	113.8 ± 27.03	38.8 ± 10.67
CQ	130.4 ± 25.97	40.2 ± 8.78	170 ± 26.47	40.2 ± 7.43
		DAY 11		
	Rearing/30 min	Grooming/30 min	Locomotion/30 min	Exploratory/5 min
CI	36.2 ± 14.04*	45.2 ± 7.36	80.2 ± 15.83*	14.2 ± 3.41*
20	19.8 ± 5.17	39 ± 8.67	38.6 ± 5.43*	8.6 ± 2.87*
40	31.6 ± 16.06	38.2 ± 14.00	76 ± 26.94	13 ± 2.59
80	52.8 ± 23.77	59.2 ± 20.08	93 ± 32.73	19.2 ± 8.51
CQ	104.6 ± 3.16 (**)	57.8 ± 16.73	131.6 ± 35.76	29.4 ± 5.68

No significance was observed on day 7 when subjected to student's t-test at each dose levels compared to control infected (CI) at ($p> 0.05$).

Table 4:
The prophylaxis test of *Harungana madagascariensis* stem bark extract on novelty-induced and exploratory behaviours in infected mice on D4 and D11. The data represent mean ± SEM of at least 5 animals. *Significance difference was observed in behaviours using student's t-test at ($p<0.05$).

		Day 4		
Treatment (mg/ kg)	Rearing/30 min	Grooming/30 min	Locomotion/30 min	Exploratory/5 min
CI	151.4 ± 13.33	52.2 ± 8.07	223.4 ± 21.44	59.2 ± 7.31
20	56.2 ± 18.08(**)	62.8 ± 20.26	75 ± 22.09(**)	43.4 ± 6.51
40	133.2 ± 34.84	57.8 ± 13.97	169 ± 35.58	47.0 ± 6.63
80	32.6 ± 7.07(**)	47.8 ±11.60	72.6 ± 13.02(**)	25 ± 3.22(**)
PYR(1.2)	165.8 ± 11.60	81.8 ± 11.06	256.8 ± 27.56	36.6 ± 1.57(**)
		Day 11		
	Rearing/30 min	Grooming/30 min	Locomotion/30 min	Exploratory/5 min
CI	105.2 ± 13.95*	47.4 ± 12.95	139 ± 24.64*	28 ± 1.05*
20	158.0 ± 23.63*	57.8 ± 10.77	195.2 ± 22.96*	28 ± 2.40
40	171.4 ± 33.18	62.4 ± 4.83	171.6 ± 14.95	26 ± 2.63*
80	62.6 ± 6.71*	59.6 ± 7.50	72.2 ± 6.8	25 ± 2.02
PYR (1.2)	187.0 ± 24.0	93.8 ± 12.69**	271.8 ± 39.04**	31.2 ± 3.87

No significance was observed on day 4 when subjected to student's t-test at each dose levels compared to control infected (CI) at ($p > 0.05$). *, **Significance was observed in rearing, exploratory and locomotion at 20 and 80mg/kg of the extract and pyrimethamine. When control infected on day 11 was compared to control infected on day 4, significance was observed in rearing at 20 and 80mg/kg, locomotion at 20 mg/kg and exploratory at 40mg/kg using student's t-test at ($p <0.05$).

Table 5:
The Nitric oxide (NO) release and Acetylcholinesterase (AchE) enzyme activity of *Harungana madagascariensis* stem bark extract (Hm) and chloroquine (CQ) on the brain cells of mice infected with and without malaria parasites. *Significant between control non-infected (CNI) vs control infected (CI), ** Significant between CI vs extract at 20, 40, 80 mg/kg and CQ 10 mg/kg. The data represent mean ± SEM of at least 5 animals.

Treatment (mg / kg)	**Amount of NO (µM)**	**AchE specific activity**
CNI	19.86 ± 1.19	0.57 ± 0.06
CI	12.89 ± 1.47*	1.85 ± 0.13*
H.M (20)	28.13 ± 2.11**	1.81 ± 0.17
H.M (40)	18.82 ± 0.85**	0.96 ± 0.37**
H.M (80)	16.28 ± 0.48	1.4 ± 0.05**
CQ (10)	11.13 ± 1.56	1.26 ± 0.14**

In curative test, significant reduction was observed in rearing, locomotion and exploration when control infected and treated groups on D11 were compared to their effects on D7 (Table 3). But when the different groups on day 11 were subjected to ANOVA followed by Benferroni t-test against infected control (CI), only CQ showed significance in rearing. In prophylactic test, there was a significant dose-dependent increase in behavioural activity of the extract and pyrimethamine (Table 4); however, there was a significant decrease in activity for the control infected when D4 was compared to D11. Table 5 indicated respectively the dose dependent increase and decrease in NO release and AchE enzyme activity of *H. madagascariensis* stem bark extract and CQ on the brain cells of mice infected with and without malaria parasites.

DISCUSSION

In malaria infection, it has been established that there is abnormal metabolism of amino acids and these circulating amino acids are the precursors for brain monoamines (serotonin, dopamine, noradrenaline and histamine) synthesis (Pardridge 1983; Enwonwu et al., 1983; Lopansri et al., 2006). Thus, malaria infection is expected to affect brain neurotransmitters and thus behaviours. The incidence of body scratching in malaria infection and chloroquine induced pruritus (Adebayo et al., 1997) has also made behaviour an important aspect of antimalarial chemotherapy. Hence, this study present the effect of ethanolic stem bark extract on novelty- induced behaviours (rearing, grooming, and locomotion) and exploratory behaviours in infected and non-infected mice using suppressive, curative, and prophylactic mode of malaria treatment.

Rearing is described as a central locomotor's excitatory behaviour (Labella et al., 1979). It is also a stereotyped (frequent and mechanical) behaviour mediated through dopaminergic system in the CNS (Ayhan and Randrup 1973; Dickinson and Curson 1982). Grooming is a behavioural component of mice and it is associated with low arousal state of the CNS which can be elicited in a novel environment (Bindra & Spinner, 1958). Exploration is also an important behaviour of mice. The increase or decrease of which reflect stimulant or depressant action of drugs. These novelty-induced behaviours may act via or can also be influenced by cholinergic or GABA neurotransmission

In control non infected mice, all the behavioural components-rearing, grooming, locomotion and exploration were consistently reduced at 80 mg/kg of the extract. This implies that depression of activities that have been extensively shown in natural product effects is directly involved in locomotive activity and is actually suppressed by the *H. madagascariensis* extract used in this study. This result showed that the extract had depressant effect on the CNS, suggesting that the cholinergic and GABA neurotransmissions in the mice brain might have been drastically inhibited (Ayhan and Randrup 1973; Dickinson and Curson 1982). This is in conformity with similar actions produced by other central nervous system depressant agents (Dorr et al., 1971), centrally-active cholinomimetic and anticholinesterase (anti-ChE) agents which decrease locomotion, exploratory and self-stimulating behaviours (Bowman and Rand 1980). In this study, *H. madagascariensis* extract tend to act as anti-ChE, lowering the activity of AChE enzyme and thereby increasing the level of cholinergic transmission in infected mice.

When control non infected mice were compared to control infected mice in suppressive test D1 and curative test on D7, rearing and grooming activities were depressed in both cases and more reduction in these activities was observed in the extract treated in control non infected group. In the first day of suppressive test, there was no significance difference in all the experimental groups in all the behavioural components. These results showed negligible effect of the parasite and the extract on novelty induced and exploratory behaviours in mice. However, in control infected mice as shown in suppressive test, there was significant reduction in locomotion and exploratory behaviours in day 4 (D4) of infection when compared to D1 which might be due to the parasite. This could be due to the loss of striatal substance P-containing neurons and induction of various neurotransmitters like histamine, 5-HT, bradykinnins, prostaglandins, substance-P in plasmodium infected mice which might contribute to their reduced locomotion (Cordeiro et al., 1983; Riley 1988; Clark et al., 2005; Iwalewa et al., 2004). In the mice treated with the different doses of the extract, there was significant reduction in grooming, locomotion and exploratory behaviours. This result might be due to the combined effect of the extract and the parasite on dopaminergic/cholinergic transmission. Depression in all the behavioural components in treated groups was observed on D4 contrary to that observed in chloroquine group which increases rearing and grooming. Chloroquine could have cleared the parasite and this may account for the increase in behaviours. This increase activity observed in CQ may be mediated by reduction in dopaminergic or cholinergic pathways (Walting 1998, Rang et al., 1999). In malaria infection, reduction in these behavioural activities can also be influenced by pro-inflammatory cytokines which might be responsible for neural dysfunction. Our result however suggests that *H. madagascariensis* extract does not totally eliminate the parasite but in addition inhibit the chemical mediators; hence, it is not as potent as chloroquine in chemo-suppressive test.

Pyrimethamine, a drug of choice for prophylaxis against malaria infection showed no significant alterations on D11 compared to D4 in all the behavioural components. This suggests that the drug did not permit the occurrence of infection, thus, pyrimethamine prevented the release of the neurotransmitters implicated in the pathogenesis of malaria infection seen in normal animals. However, when D11 was compared to D4 in control infected group, there was reduction in rearing, locomotion which may be associated with neuronal dysfunction in the pathogenesis of malaria (Clark et al., 2005). There was reduction in exploration behaviour which might be mediated via cholinergic neurotransmission. In the prophylactic treated group with the extract, there was increase in rearing and locomotion from D4 to D11. This result suggests that the extract prevent the multiplication of parasite like the positive control drug pyrimethamine, thereby reducing/preventing the release of autacoids which contribute to the symptoms of behavioural changes in malaria. This behavioural result corroborates the effective prophylactic chemotherapy of the extract in earlier research findings of Iwalewa et al., (2008).

It then points to the fact that in prophylaxis, the extract also modulates the action of neurochemicals to enhance the behavioural activities of the animals bearing in mind that in non-infected treated mice, there was decrease in all the behavioural parameters. This then shows that the extract could be acting through selective cytotoxicity against the malaria parasite. This is buttressed by the work of Muthaura et al., (2007) who showed that *H. madagascariensis* evaluated among five plants in Kenya against malaria has the lowest selectiveindex of 11.8 as a sign of toxicity in malaria in vitro. Likewise Iwalewa et al., 2007 has indicated that the mechanism of action of *H. madagascariensis* could be by cytotoxicity through the release of nitric oxide. Nitric oxide (NO) is implicated in some aspects of the

pathogenesis of severe malaria (Grau et al., 1994; Clark et al., 1994) and the significantly reduced plasma arginine observed in malaria patients (Enwonwu et al., 1999) could be due to increased utilization of this amino acid by the nitric oxide synthase (NOS) pathways. In this study, NO content in control

infected mice was lowered, however, NO was significantly increased in the treated groups with the extract. It is therefore suggested that this could be the patternthrough which our extract exhibits its antimalarial activity and reverse the behaviour in this study.

Considering these results, we suggest that NO may modulate motor behaviour, probably by interfering with NO and cholinergic neurotransmission in the brain (Del Bel et al., 2005). The results obtained from this study are a pointer to future study of how *H. madagascariensis* could be implicated in alzheimers disease in conjunction with malaria infection.

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