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Research article

Antidepressant and Anxiolytic Potentials of Dichloromethane Fraction from *Hedranthera barteri*

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ABSTRACT: The dichloromethane fraction of *Hedranthera barteri* (DMHBR), a common medicinal plant, was investigated in animal models of depression and anxiety in mice. Graded doses (25-200mg/kg p.o. bw) of DMHBR reduced the immobility time with significant effects produced by 50mg/kg (43.7%), 100mg/kg (45.6%) and 200mg/kg (31.5%) in the tail suspension test (TST) and by 100mg/kg (66.3%) in forced swimming test (FST), indicating a possible antidepressant-like activity when compared with standard antidepressant drug, imipramine. Furthermore, a diminution in the anxiety response was also observed against elevated plus maze and light dark tests, which signify its anti-anxiety activity when compared with standard anxiolytic drug, diazepam. Moreover, DMHBR has no significant effects on both the motor coordination of the mice in the rota rod test and the sleeping time in the pentobarbitone-induced sleeping time test. These results show that DMHBR has significant neuropharmacological activity as an antidepressant and anxiolytic activity.

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Keywords: Anxiety, depression, *Hedranthera barteri*, imipramine, diazepam.

INTRODUCTION

A number of the world population suffers from depression and anxiety at some time during their life, and these conditions are the most prevalent psychiatric disorders known. About 450 million people suffer from a mental or behavioral disorder but only a small minority of them receives even the most basic treatment (WHO, 2001). This amounts to 12.3% of the global burden of disease which may rise to about 15% by 2020 (Reynolds, 2003). With this alarming anticipated rise, World Health Organization envisaged that depression will become the second leading cause of premature death or disability worldwide by the year 2020 (WHO, 2001). Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the extent of improvement is still disappointing, coupled with the various physiological side effects and tolerance on chronic treatment.

Drugs prescribed for neuropsychiatric disorders have more side effects than they are efficacious. In a scenario like this, we need drugs with lesser side effects. Hence ayurveda has recently become the drug of choice and investigations have been extended for the search of novel and better tolerated molecules from plant sources. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research has progressed constantly, demonstrating the pharmacological effectiveness of

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different plant species in a variety of animal models (Zhang, 2004).

Hedranthera barteri is a shrub that has been established scientifically for the management of certain nervous system challenges like dizziness (Thomas, 1967), pain and inflammation with COX-2 inhibiting activity, in-vitro (Ainslie, 1937; Chukwujekwu et al, 2005; Onasanwo and Elegbe, 2006; Onasanwo *et al.*, 2008). Despite these effects on the aspect of the nervous system, there has not been any report of its effect on anxiety and depression which are also pathological conditions of the nervous system. Therefore, the objective of the present study was to evaluate the antidepressant and anxiolytic potentials of *Hedranthera barteri* in different models in mice.

MATERIALS AND METHODS

Animals: Albino male Swiss mice (22-25g) obtained from National Animal Laboratory Centre of Central Drug Research Institute, Lucknow were used in the study. The mice were kept at constant temperature (22±2^{o}C) and 12-h light/12-h dark. Mice were fed standard laboratory food (Hind Lever diet pellets) and water was given *ad libitum*. Each animal was used once in the behavior tests. The experimental protocols for this study were approved by the Institutional Ethical Committee following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which complies with International norms of Indian National Science Academy (INSA).

Plant description: *Hedranthera barteri* (Apocynaceae) is a shrub found in damp situations of the closed-forest in South Nigeria, Ghana, North/West Cameroon, Congo Brazzaville and other parts of the world. It has large white tubular flowers, with fragrant scent and contains white latex that does not coagulate. The shape of the free bi-carpelate fruits evokes the bawdy Ijaw name meaning "goat testicles" and also the Yoruba name "dog's penis" in Nigeria (Dalziel, 1995).

Phytochemical screening: Preliminary phytochemical screening of the powdered root was performed for the presence of alkaloids, cardenolides, flavonoids and Saponins (Trease and Evans, 1995).

Preparation of extract: The roots of *Hedranthera barteri* (HB) were purchased from the Herbarium Department, Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and authenticated voucher specimen (FHI-108375), prepared by Mr. Felix Usani, a staff of FRIN, was deposited there. The roots of HB were dried under shade during the harmattan season in Nigeria (November-December) and 2kg of the dried root powder were percolated with hexane for 48hrs, after which the hexane fraction (HXHBR) was filtered off through a separate funnel and the solvent was removed at 40^{0}C under reduced pressure in a rotavapor. The marc was dried under shade and subjected to further extraction with the dichloromethane. The same procedure was employed to extract the dichloromethane fraction (DMHBR). The yields for hexane and dichloromethane extracts of HB were 13.1% and 18.7% respectively. The semi-solid samples of the extract were stored at 4^{0}C until when needed. The extracts were prepared as suspensions using 2.5% tween 20/distilled water, and were administered 45mins before each experimental model.

Drugs and treatment schedule: Imipramine, IMP (60mg/kg) was used as the standard drug for depression (Sigma, USA) while diazepam, DZP (1.5 mg/kg) obtained from Ranbaxy Laboratories Ltd. India, was used as standard drug in anxiety. All compounds were dissolved in 0.9% physiological saline and freshly prepared. Compounds were administered per orally at a rate of 0.1 ml/10 g.

Open field test: Gross open field activity was studied using Digiscan Infrared Photocell system [Test Box model: RXYZCM (16 TAO); Omnitech Electronics, Columbus, Ohio] in (42x42x30)cm plexiglass arenas, fitted into infrared beam containing metallic grid. Activities of animals were observed by the interruption of infrared beams. Prior to the experiment, animals both treated and the controls, were habituated in the Test Box for 15mins. After the initial habituation process, the activity of the treated and control animals were studied for 2minutes at regular intervals of 15 minutes for duration of 1 hour. DMHBR was administered one hour prior to the experiment.

Depression Models

Tail suspension test: The tail suspension test (TST) was performed according to the method described by Steru *et al*, (1985). The mice were individually suspended 60 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. Immobility duration was recorded for the last 5 minutes during 6 minutes by observers blind to the treatment conditions. Mice were considered immobile only when they hung passively and were completely motionless. Single administrations (p.o.) of DMHBR extract (25, 50, 100

and 200mg/kg) and IMP (60mg/kg p.o.) was given one hour prior to test.

Forced swim test: The forced swim test (FST) is the most widely used pharmacological *in vivo* model for assessing antidepressant activity and was performed according to the method of Porsolt *et al*, (1977). The apparatus consists of a clear plexiglass cylinder (20cm high by 12cm diameter) filled to a 15cm depth with water (24 ± 1°C). Animals were divided into groups of six animals each. One of the groups received only saline treatment. In the pre-test session, every animal was placed individually into the cylinder for 15mins, 24hrs prior to the 6mins swimming test, in which the duration of immobility was recorded for the last 5mins. Oral administration of the graded dose of DMHBR (25, 50, 100 and 200mg/kg) and IMP (60mg/kg p.o.) was administered one hour prior to final swimming test session. The period between when the mouse was immersed and when no further attempts to escape was made (apart from the movements necessary to keep its head above the water) was recorded as the immobility time.

Anxiety model

Light-Dark Test: The apparatus consisted of a Plexiglas box with two compartments (20cm × 20cm each), one of which was illuminated with a white light while the other remained dark. Each animal was placed at the junction of the light dark, facing the illuminated compartment. The time spent in illuminated places, as well as the number of entries in each space, was recorded for 5minutes (Young, 1991). After each test, the box was carefully cleaned up with a wet tissue paper (10% ethanol solution). Single administration (p.o.) of DMHBR (50, 100 and 200mg/kg) and DZP (1.5mg/kg p.o.) was given one hour prior to test.

Elevated plus Maze: This test has been widely validated to measure anxiety in rodents (Lister, 1987). This apparatus was made of stainless steel and consisted of two open arms and two closed arms (30cm × 5cm) with 25cm walls. The arms extended from a central platform (5cm × 5cm). The maze was elevated 38.5 cm from the room floor. All the four arms consist of infrared beams fitted at regular distance. Mice were treated with DMHBR (50mg/kg p.o.), which was found to be effective in the light-dark model and DZP (1.5mg/kg p.o.) one hour prior to the experiment. Each animal was placed at the center of the maze, facing one of the open arms. The time spent in enclosed and open arms was recorded in 5mins. The movement of animals across the arms is calculated by interruption of beams which was analyzed by Maze tracking software (M/s Columbus Instruments, USA). After each test, the maze was carefully cleaned up with 10% ethanol solution.

Rota rod test in mice: Rota rod test is commonly used for evaluation of neurotoxicity or neurological deficit in mice treated with various plant extracts used in the study. The protocol was used as described by Dunham and Miya (1957) and studied in the Rotamex 4/8 apparatus (M/s Columbus Instruments, USA). Basically, the rota rod consists of a rod which is coated with rubber or polypropylene foam to provide friction and to prevent animals from slipping off the rod. The distance between the rod and floor is kept 15cm to avoid intentional jumping of mice. The rod is driven by a motor and the rotational speed can be regulated which is maintained at 8rpm in our study. Animals were trained on the rota rod for duration of 2mins/trial, with 4trials/day for two days. On the third day, mice were given trials before and after treatment with DMHBR (200mg/kg p.o.) to evaluate any neurotoxic effects.

Pentobarbitone-induced sleeping time test: The animals were randomly divided into six groups consisting of six mice each. The test groups received various doses of DMHBR (25, 50, 100 and 200mg/kg p.o.) while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (50mg/kg i.p.) was administered to each mouse to induce sleep. The animals were observed for the duration of sleep which depicts the time between the loss and recovery of righting reflex (Ramirez *et al.*, 1998).

Statistical analysis

The results were expressed as mean±S.E.M. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by Newman–Keul's multiple comparisons test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Antidepressant-like activity evaluation

Effects of DMHBR on the tail suspension test (TST): The effects of the dichloromethane fraction of *Hedranthera barteri* root extract (DMHBR) and imipramine (IMP) on active behaviors in the TST of mice are shown in Table 1. Oral administration of various doses of DMHBR (25, 50, 100 and 200mg/kg)

were used to assess the extent of immobility in mice exposed to tail suspension test (TST).

Table 1:
Effect of DMHBR and imipramine on tail suspension model in mice.

Group	Dose (mg/kg)	Duration of immobility (sec)
Control	-	198.3±18.1
DMHBR	25	153.5±23.4
DMHBR	50	111.7±8.3**
DMHBR	100	108 ±23.6**
DMHBR	200	135.8±5.2*
Imipramine	60	30.2±7.2***

*Data represent means ± S.E.M. of 8 mice during the 5-min test session. Comparisons were made by using a one-way ANOVA followed by post hoc Newman–Keuls's test: *p< 0.05, **p< 0.01, ***p < 0.001 compared with control group.*

Table 2:
Effect of DMHBR and imipramine on forced swim model in mice

Group	Dose (mg/kg)	Duration of immobility (sec)
Control	-	176.8±18.8
DMHBR	25	123.7±12.5
DMHBR	50	113.0±23.5
DMHBR	100	59. 7±4.6**
DMHBR	200	123.8±7.9
Imipramine	60	12.8±4.3***

*Data represent means ± S.E.M. of 8 mice during the 5minute test session. Comparisons were made by using a one-way ANOVA followed by post hoc Newman–Keuls's test: **p< 0.01, ***p < 0.001 compared with control group.*

DMHBR and IMP induced significant diminution of immobility time [Vehicle, 198.33±18.1; DMHBR 50mg/kg, 111.7±8.3 (p< 0.01); DMHBR 100mg/kg, 108±23.6 (p< 0.01); DMHBR 200mg/kg, 135.8±5.2 (p< 0.05); and imipamine, 60mg/kg, 30.2±7.2 (p< 0.05)] as compared with the control. There was no significant difference between the effect of the various doses of the DMHBR and that observed with imipramine on the immobility time group when the mice were exposed to the TST.

Effects of DMHBR on the forced swim test (FST): The effects of the dichloromethane fraction of *Hedranthera barteri* root extract (DMHBR) and imipramine (IMP) on active behaviors in the FST of mice are shown in Table 2. Out the four doses of DMHBR (25, 50, 100 and 200mg/kg) administer, only 100mg/kg significantly reduced the immobility time [Vehicle, 176.8 ± 18.8; DMHBR 100mg/kg, 59.67±4.6 (p< 0.01)] compared to negative control values. Also, imipramine significantly decreased the immobility time [imipamine, 60mg/kg, 12.75±4.3 (p< 0.001)] during the 5minute test session. There was no significant difference between the effects of the DMHBR (100mg/kg) and that observed with imipramine on the immobility time when the mice were exposed to the FST.

Table 3:
Effects of DMHBR and diazepam on the light-dark model in mice.

Group	Dose (mg/kg)	Time spent in the light chamber (sec)
Control	-	64.8±12.7
DMHBR	50	175.7±26.2**
DMHBR	100	131.0±8.3*
DMHBR	200	127.7±19.7*
Diazepam	1.5	189.1±4.8***

*Data represent means ± S.E.M. of 8 mice during the 5minute test session. Comparisons were made by using a one-way ANOVA followed by Newman–Keuls's test as the post hoc: *p< 0.05, **p< 0.01, ***p < 0.001 compared with control group.*

Table 4:
The effects *of DMHBR* and diazepam on the time spent in open arms of the elevated plus-maze in mice.

Group	Dose (mg/kg)	Time spent in the closed arm (secs)	Time spent in the opened arm (secs)
Control	-	287.6 ± 6.4	11.5±6.3
DMHBR	50	209.9 ± 46.2	87.7±44.7
DMHBR	100	172.2 ± 14.1**	123.9±42.9**
DMHBR	200	205.7 ± 29.1	87.5±24.9
Diazepam	1.5	185.2 ± 13.2**	110.6±14.5**

*Data represent means ± S.E.M. of 8 mice during the 5minute test session. Comparisons were made by using a one-way ANOVA followed by Newman-Keuls's test as the post hoc: *p< 0.05, **p< 0.01, ***p < 0.001 compared with control group.*

Anxiolytic-like activity evaluation
Effects of DMHBR on the light-dark test: The oral administration of graded doses of DMHBR in mice significantly increased the time spent by mice on the illuminated side of the light-dark chamber [Vehicle,

64.8 ±12.7; DMHBR 50mg/kg, 175.7 ± 26.2 ($p < 0.01$); DMHBR 100mg/kg, 131.0±8.3 ($p < 0.05$); DMHBR 200mg/kg, 127.7±19.7 ($p < 0.05$)] similar to the effect observed in mice treated with diazepam, DZP [Vehicle, 64.8 ±12.7; DZP 1.5mg/kg, 189.1±4.8 ($p < 0.001$)] as shown in Table 3.

Effects of DMHBR on mice in the elevated plus maze test (EPM): The oral administration of different doses of DMHBR in mice exposed to the EPM test increased the time that mice spent in open arms [Vehicle 11.5±6.3; DMHBR 100mg/kg, 123.9 ± 42.9; p< 0.01] compared with the negative control (Table 4). Moreover, this result was not significantly different from the group treated with diazepam [Vehicle,; DZP 1.5mg/kg, 110.6±14.5 (p< 0.01)].

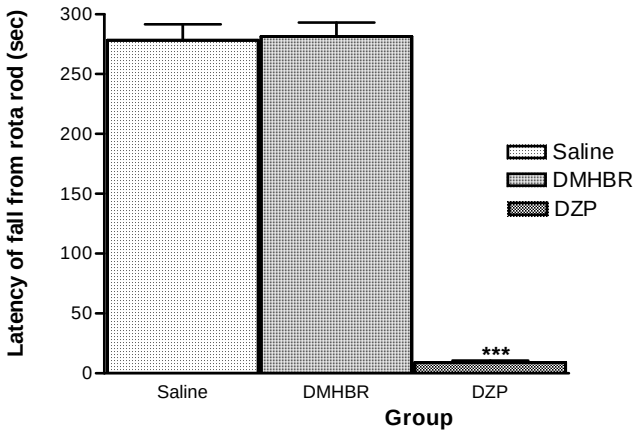

Figure 1:
Effect of DMHBR and diazepam on latency to fall off the rota rod. *Data represent means ± S.E.M. of 8 mice. Comparisons were made by using a one-way ANOVA followed by Newman-Keuls's test as the post hoc: ***p < 0.001 compared with control group.*

Effect of DMHBR on spontaneous locomotor activity: In the rota rod test, DMHBR-treated mice showed no significant motor performance alterations with the dose of 200mg/kg (281.5±11.7sec) compared to control (278.3±13.4sec). Diazepam, a known central nervous system depressant at a dose of 10mg/kg p.o., significantly reduced the locomotor activity of the mice (9.3±1.3sec, $P < 0.001$) as shown in figure 1.

Effect of DMHBR and diazepam on pentobarbital sleeping time: The extract (25-200mg/kg, p.o.) showed no significantly duration of pentobarbital sleeping time in rats. Diazepam prolonged the duration of pentobarbital sleeping time in rats as shown in Table 2.

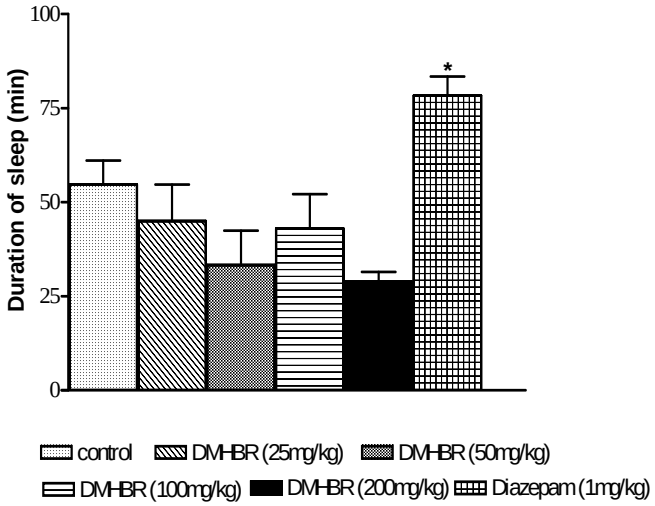

Figure 2:
Effect of DMHBR and diazepam on pentobarbitone-induced sleeping time in mice. Data represent means ± S.E.M. of 6 mice. Comparisons were made by using a one-way ANOVA followed by Newman-Keuls's test as the post hoc: *p< 0.05 compared with control group.

DISCUSSION

The present study investigated the behavioral effects of the dichloromethane fraction from *Hedranthera barteri* root (DMHBR) in mice and rats. To the best of our knowledge and for the first time, this research work on *Hedrathera barteri* and especially, DMHBR produced significant antidepressant-like effects. When assessed in Tail Suspension Test (TST), the fraction was able to induce antidepressant-like effects after oral administration of varying doses of DMHBR with the 100mg/kg dose showing the highest immobility. Furthermore, antidepressant-like potentials of DMHBR was explored using forced swimming test (FST). It exhibited antidepressant-like effects with decrease in the immobility time which is accompanied with the increase in swimming time. These models were used because the two most widely used animal models for screening new antidepressant drugs have been reported to be the forced swimming and tail suspension tests (Porsolt et al., 1977; Steru et al., 1985).

The efficacies of these two models of depression have been reviewed. Petit-Demouliere *et al.,* (2005) reviewed FST with mice and concluded that the assay has good reliability and predictive validity. Likewise, Steru et al. (1985) described TST as another test as primary screening procedure which induces a state of despair in animals like that as in FST. The main advantages of these procedures are the use of a simple,

objective test situation, the concordance of the results with the validated "behavioral despair" test. (Porsolt et al., 1977) and the sensitivity to a wide range of drug doses (Steru et al., 1985; Cryan et al., 2005). These behavioral effects of DMHBR are similar to data obtained by other investigators with classical antidepressants drugs, such as imipramine (or others tricyclic), monoamine oxidase inhibitors and selective serotonin re-uptake inhibitors agents (Porsolt et al., 1977; Borsini and Meli, 1988; Petit-Demouliere et al., 2005; Cryan et al., 2005).

The antidepressant effects of DMHBR in FST and TST were more prominent at 100mg/kg when compared to higher or lower doses of the same fraction. The prominent significant antidepressant effects at dose 100mg/kg could be due to strong and effective concentration of the active constituent at that dose compared to other doses of the plant extract. It has been reported that swimming behavior is sensitive to serotoninergic agents, such as the selective serotonin reuptake inhibitor (SSRI), fluoxetine [Cryan and Lucki, 2000, Cryan *et al.,* 2002, Detke *et al.,* 1995]. Based on the these findings, it can be suggested that the DMHBR which is able to decrease the immobility time and increases swimming behavior in the mice exposed to these paradigms can exert its effect through a mechanism similar to that of the fluoxetines via the serotonin system. Moreover, imipramine belongs to the class of tricyclic antidepressant drugs which blocks the reuptake of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) into their respective neurons. Hence, DMHBR can also mediate its activity through the same mechanism as that of imipramine. However, the precise mechanism underlying DMHBR activity still required further investigations.

Furthermore, this work demonstrated that the administration of different doses of DMHBR in mice was able to induce anxiolytic effects, without significantly modifying the spontaneous motor activity. This animal model, especially with mouse, has been considered to be one of the most widely validated tests for assaying sedative and anxiolytic substances (Pellow *et al.,* 1985). The light-dark and elevated plus maze tests were used to access the anxiolytic potentials of DMHBR. In the light-dark test, 50mg/kg dose showed the most prominent anxiolytic-like properties by spending the highest time in the light chamber. This corroborate the suggestion that the time mice spent in the illuminated side of the light-dark chamber is the most useful and consistent parameter of anxiety (Young and Johnson, 1991). It is worth-noting that lack of dose-dependent effect could be explained based on the biological variability and secondary metabolites interaction present in DMHBR (Maribel *et al.,* 2006).

In the elevated plus maze test, only 100mg/kg DMHBR was able to show a significant anxiolytic-like properties with the time the mice spent at the opened arm being more than the observed time with the mice that were given diazepam. The lower and higher doses did not produce significant changes. The possible explanation for this insignificant activity might partly be due to the difference in the concentration of constituents in DMHBR. It is possible that each chemical constituents of the fraction exhibited the biological activity influencing on the neurobehaviors involving depression activity. It could also be explained that the increasing concentration of some constituents might have masked the effect of active constituent which shows anti-depressant effect.

In the spontaneous locomotor activity experiment, DMHBR (200mg/kg) had no significant effect on the motor co-ordination when compared to the control group. This experiment was carried out to explore possible neurotoxicity of DMHBR. All the mice stayed on the rota-rod for more than 250secs, suggesting that DMHBR showed no neurotoxic effect. Diazepam treated group showed a sedative effect as expected. The central activity of DMHBR without depression was confirmed by its insignificance in the duration of sleep in all the doses given to the mice. Hence, the analgesic property of *Hedranthera barteri* reported earlier (Onasanwo and Elegbe, 2006; Onasanwo *et al.,* 2008) might not involve the depression of the nervous system.

From the present study, it can be concluded that dichloromethane fraction of *Hedranthera barteri* (DMHBR) shows significant psychotherapeutic effects as antidepressant and anxiolytic agents with intact motor coordination. This might have been attained through its influence on the levels of monoamines. This research work has eliminated the involvement of neurotoxicity in the use of *Hedranthera barteri* for pharmacotherapy in anxiety and depression. Detailed laboratory analysis is required for a definitive conclusion and isolation of the major active secondary metabolite responsible for these therapeutic actions. So, a potent antidepressant and anxiolytic may emerge from *Hedranthera barteri.* Since the extract shows potent anxiolytic and antidepressant effects nearly at the same dose range hence it could also be used in treatment of mixed anxiety and depressive syndrome.

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