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Effects of dimethyl fumarate in murine models of depression and anxiety

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Abstract

Depression and anxiety are psychiatric disorders, which are leading causes of disability and often accompany chronic diseases. Increased oxidative stress occurs in both disorders. This study investigated the effects of dimethyl fumarate (DMF) in animal models of depression and anxiety. Different groups of mice were treated with either the vehicle, 50, 100 mg/kg DMF or imipramine and subjected to either the forced swim test (FST) or the tail suspension test (TST). Another set of mice were treated daily with either the vehicle, DMF 50 and 100 mg/kg and imipramine for two weeks and subjected to either the FST or TST. Thereafter, animals were sacrificed; whole brains isolated and brain catalase levels assayed. The same procedure was followed for evaluation of anxiolytic property of DMF using the staircase and hole-board tests as test indices and diazepam as the reference drug. In the test for depression, 50 and 100 mg/kg DMF significantly ($p<0.05$) reduced periods of immobility in both the FST and TST after acute and chronic drug administration; and significantly ($p<0.05$) increased brain catalase levels. In the test for anxiolysis, both doses of DMF did not produce significant changes in the staircase test indices following acute and chronic drug treatment. However, low dose DMF -50 mg/kg significantly increased ($p<0.05$) the number of head dips in the hole-board test post chronic drug treatment; both doses increased levels of catalase in the brain. DMF exhibited antidepressant activity and anxiolytic properties and increased levels of catalase in the brains of mice.

Keywords: Catalase; Forced swim test; Staircase test; Antioxidants; Depression

INTRODUCTION

Depression and anxiety are psychiatric disorders, which are leading causes of disability. While depression is estimated to be a major contributor to overall global burden of disease, anxiety disorders affect the individual performance of day-to-day tasks and presents a high cost for public healthcare all over the world [1-3]. Additionally, anxiety and depression often accompany chronic diseases such as multiple sclerosis, diabetes, ischaemic and haemorrhagic strokes [4-8].

The brain is particularly sensitive to oxidative damage resulting from free radical production due to its capacity to consume disproportionately large quantities of oxygen and the presence of high amounts of oxidizable poly unsaturated fatty acids [9,10]. Oxidative stress is thus implicated in several disorders of the brain such as neurodegenerative disorders, depression, anxiety and neuropsychiatric ailments [9,11-13]. Dimethyl fumarate (DMF), a fumaric acid ester currently used in the management

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of remitting relapsing multiple sclerosis, is an anti-oxidant with anti-inflammatory and neuroprotective properties in the CNS [14,15].

In this study, we sought to evaluate (i) the effects of DMF in behavioural indices using murine models of depression and anxiety (ii) levels of catalase in the brains of mice following chronic treatment with DMF.

EXPERIMENTAL

Animals. Swiss albino male mice procured from the University of Benin Animal House, Benin City, Edo State were used for the study. They were kept in plastic cages at the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin City. They were maintained under standard controlled environment and were allowed free access to feed (Top feeds® Growers Mash, Super-Deluxe Animal Feed Mills Co. Ltd, Nigeria) and clean water *ad libitum*. Handling of the animals was done according to standard protocols for the use of laboratory animals of the National Institute of Health [16] and ethical approval was obtained from the institutional Ethics and Research Committee.

Chemicals and drugs. Dimethyl fumarate 97% and dimethyl sulphoxide were purchased from Sigma Aldrich, USA; diazepam was purchased from Swipha Pharmaceuticals, Lagos, Nigeria while Imipramine was purchased from Novartis, Basel, Switzerland. Chloroform 99%, sodium chloride, hydrogen peroxide, disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from JHD Chemicals, Guangdong, China.

Acute study for antidepressant activity

Forced swim test. The method of Porsolt *et al.* [17] with some modifications was used. Twenty-four male mice weighing 18-26 g were randomly divided into four groups of six animals each. The first group served as control and was given a solution of dimethyl sulphoxide and distilled water intra-peritoneally. The second and third group received graded doses (50 mg/kg and 100 mg/kg) of dimethyl fumarate dimethyl sulphoxide/distilled water solution intra-peritoneally respectively, while the fourth group received aqueous solution of imipramine (15 mg/kg) also intra-peritoneally. Thirty minutes post administration, each mouse was placed in a transparent cylindrical container with a diameter of 26.1 cm and height of 24.8 cm containing water at a depth of 15 cm and a temperature of 25°C for five minutes. The period of immobility in the last four minutes of the experiment was cumulatively recorded by observers blinded to treatment. After each test, the mice were properly dried before they were returned to their cages. A sieve was also used to remove animal droppings from the water and the water was changed intermittently.

Tail suspension test. The method of Steru *et al.* [18] with some modifications was used. Twenty-four male mice weighing 18-26 g were randomly divided into four groups of six mice each. The first group served as control and was given a solution of dimethyl sulphoxide and distilled water intra-peritoneally. The second and third group received two doses - 50 and 100 mg/kg- of dimethyl fumarate in a solution of dimethyl sulphoxide and distilled water intra-peritoneally respectively while the fourth group received aqueous solution of imipramine at a dose of 15 mg/kg intra-peritoneally. Thirty minutes post drug administration, each mouse was suspended form a tabletop at a height of 67.6 cm by approximately one inch from the tip of the tail using a masking tape. The period of immobility was scored by observers blind to treatment.

Acute study for anxiolytic activity

The staircase test. The staircase test was conducted according to the method of

Simiand *et al.* [19]. Twenty-four male mice weighing between 17-32 g were randomly divided into four groups of six animals each. The first group served as control and were given dimethyl sulphoxide solution intra-peritoneally. The second and third group received 50 and 100 mg/kg dimethyl fumarate intra-peritoneally respectively, while the fourth group received diazepam at a dose of 1 mg/kg intra-peritoneally. Thirty minutes post drug administration, each mouse was placed in the staircase with its back to the staircase for five minutes. The number of upward climbs, episodes of grooming and rearing and number of leans on the wall were scored by observers blind to the treatment [19]. The apparatus was properly cleaned and wiped with 70% alcohol solution after each test.

The hole-board test. Following the method of Boisseir *et al.* [20], twenty-four male mice weighing between 17-32 g were randomly divided into four groups of six animals each. The first group served as control and were given dimethyl sulphoxide solution (vehicle) intra-peritoneally. The second and third groups received 50 and 100 mg/kg dimethyl fumarate intra-peritoneally respectively, while the fourth group received diazepam at a dose of 1 mg/kg intra-peritoneally. Thirty minutes post drug administration each mouse was placed at the centre of the hole-board for five minutes and the number of head dips, episodes of rearing and grooming were recorded by unbiased observers [20]. After each test, the apparatus was properly cleaned and wiped with 70% alcohol solution.

Chronic study for antidepressant activity. Fifty-six male mice weighing between 18-25 g were randomly divided into four groups of fourteen animals each. Mice in the first group served as control and were treated with the vehicle intra-peritoneally. The second and third group received 50 and 100 mg/kg of dimethyl fumarate in a solution of dimethyl sulphoxide and distilled water intra-peritoneally respectively, while the fourth group received aqueous solution of imipramine at a dose of 15 mg/kg intra-peritoneally. The drugs were administered to the mice daily for two weeks. After 2 weeks of daily drug administration, the mice were divided into two groups of four subgroups each and one group was subjected to the forced swim test while the other group was subjected to the tail suspension test using similar protocol as with the acute study.

Chronic Study for anxiolytic activity. Sixty-four male mice weighing between 18-25 g were randomly divided into four groups of sixteen animals each. The first group served as control and was given a solution of dimethyl sulphoxide and distilled water intra-peritoneally. The second and third group received graded doses (50 and 100 mg/kg) of dimethyl fumarate in a solution of dimethyl sulphoxide and distilled water intra-peritoneally respectively while the fourth group received aqueous solution of diazepam at a dose of 1 mg/kg intra-peritoneally. The drugs were administered to the mice daily for two weeks. Two weeks after drug treatment, the mice were divided into two groups of thirty-two animals per group. Animals in each group were subjected to the either the staircase or hole-board test using similar protocol as with the acute study.

Biochemical analysis: measurement of catalase activity. Twenty-four hours after the various neurobehavioural evaluations, animals were anaesthetized with 99% chloroform, euthanized and the whole brains were carefully isolated. Whole brain were weighed, transferred into clear bottles, homogenized, centrifuged and the supernatants layers collected and stored. Evaluation of catalase activity was done using the method of Clairborne [21]. A solution of disodium hydrogen phosphate (5.5 g), sodium dihydrogen phosphate (1.6 g) and sodium chloride (4.5 g) in 1000 ml of distilled water was prepared and the pH of the resulting phosphate buffer was adjusted to 7.0 with a

few drops of hydrochloric acid. Solutions of phosphate buffer (1.95 ml), 1 ml of hydrogen peroxide and 0.05 ml of supernatant layer of brain homogenate were transferred into previously labelled bottles and absorbance of each assay sample was measured at 240 nm using a UV/VIS spectrophotometer. A blank, containing 2 ml of buffer and 1ml of hydrogen peroxide only was used as the control solution.

Statistical analysis. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test using Sigma Stat® version 11. A difference was considered significant at $p< 0.05$. The results are presented as mean ± standard error of mean (SEM).

RESULTS

Behavioural evaluation after acute drug administration

Forced swim and tail suspension tests. In the acute phase of the study, both doses of DMF reduced periods of immobility in both the forced swim and tail suspension tests, this was significantly ($p<0.05$) different from vehicle treated animals but not different from imipramine treated animals. Data is presented in Figs 1 & 2.

Staircase and hole-board and tests. In the acute phase of the study, both doses of DMF did not produce significant changes in indices observed in the staircase test-upward climb, leans on the wall and episodes of rearing and grooming when compared to the vehicle treated animals. Low dose DMF increased number of head dips but this was not significantly different from the vehicle or imipramine treated groups, no significant difference was observed in number of episodes of rearing and grooming scored the hole-board test. Data is presented in Figs 3 & 4.

Behavioural evaluation after chronic drug administration

Forced swim and tail suspension tests. In the chronic phase of the study, both doses of DMF reduced periods of immobility in both the forced swim and tail suspension tests, this was significantly ($p<0.05$) different from vehicle treated animals but not different from imipramine treated animals. Data is presented in Figs 5 & 6.

Staircase and hole-board and tests. In the chronic phase of the study, both doses of DMF did not produce significant changes in indices observed in the staircase test-upward climb, leans on the wall and episodes of rearing or grooming when compared to the vehicle treated animals. Though there were also no significant changes in episodes of rearing and grooming scored in the hole board test, 50 mg/kg DMF significantly increased the number of head dips. Data is presented in Figs 7 & 8.

Estimation of brain catalase levels. In the chronic phase of the study, both doses of DMF increased levels catalase in the brain. Data is presented in Figs 9 & 10.

DISCUSSION

In this study, treatment with DMF (i) reduced periods of immobility in both the forced swim and tail suspension tests following both acute and chronic drug administration (ii) improved number of head dips post chronic drug treatment (iii) improved brain catalase levels following chronic drug administration. The forced swim test is a rodent screening test for potential human antidepressant drugs. It is based on the rodent's persistence in trying to escape from a stressful stimulus. The rodent is put in water, which induces a depressive state and thus will exhibit longer periods of immobility. Animals treated with antidepressants will swim actively and exhibit shorter periods of immobility [22].

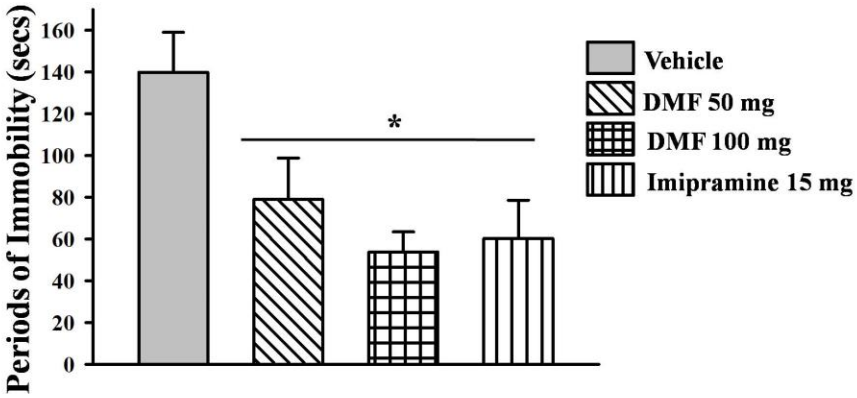

Fig 1: Statistical analysis of periods of immobility following acute drug administration in the forced swim test. Both doses of DMF reduced periods of immobility. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=6 per group

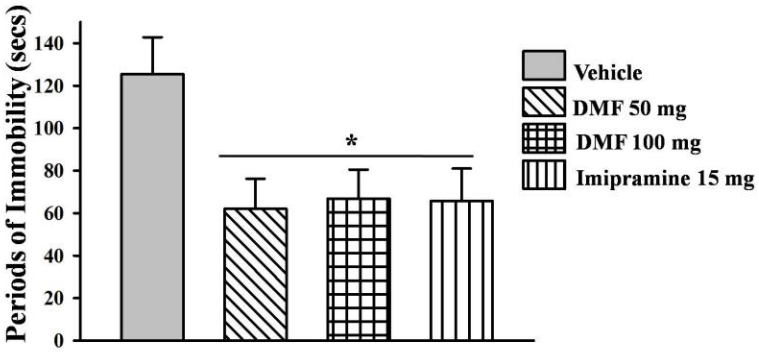

Fig 2: Statistical analysis of periods of immobility following acute drug administration in the tail suspension test. Both doses of DMF reduced periods of immobility. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=6 per group

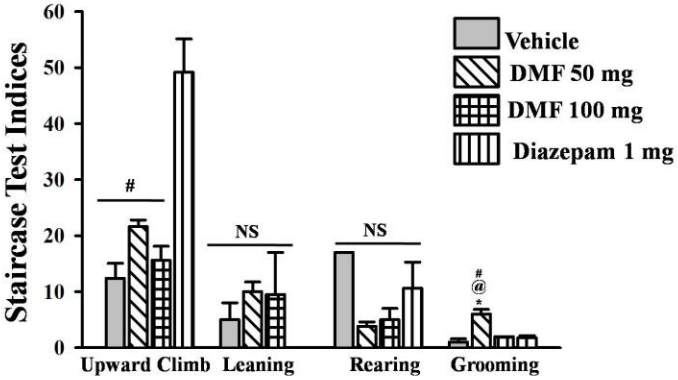

Fig 3: Statistical analysis of staircase test indices. Both doses of DMF did not increase number of upward climbs, or reduce leaning or episodes of rearing but low dose, increased episodes of grooming. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle, #p<0.05 compared to imipramine, $^{@}$p<0.05 compared to DMF 100 mg/kg; n=6 per group

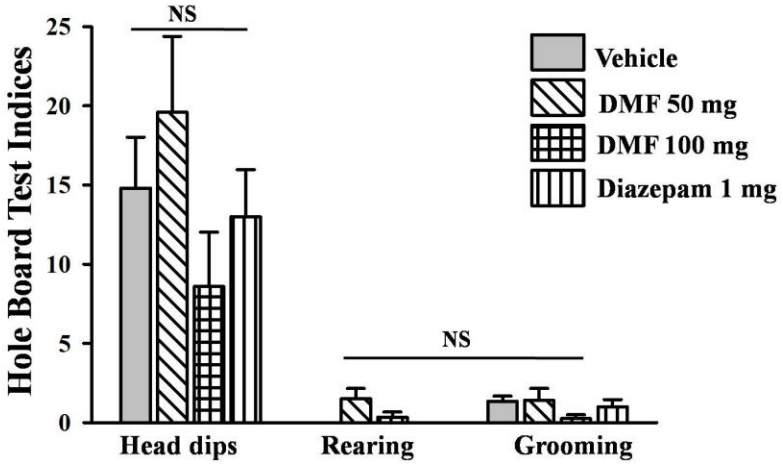

Fig 4: Statistical analysis of hole-board test indices. Both doses of DMF did not increase number of head dips or reduce episodes of rearing or grooming. Data is expressed as mean ± SEM. NS, not significant; n=6 per group

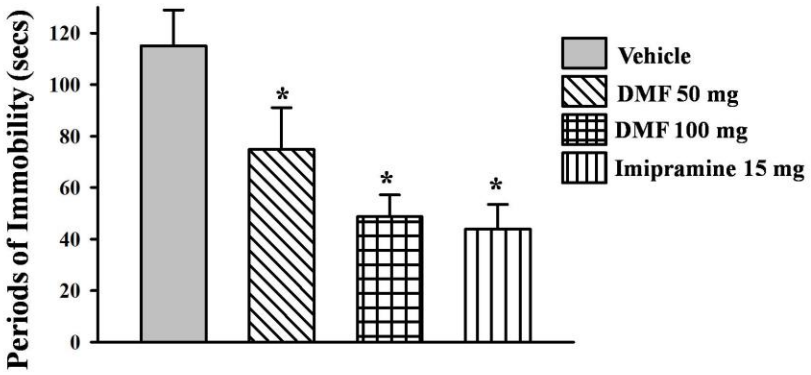

Fig 5: Statistical analysis of periods of immobility following chronic drug administration in the forced swim test. Both doses of DMF reduced periods of immobility. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=8 per group

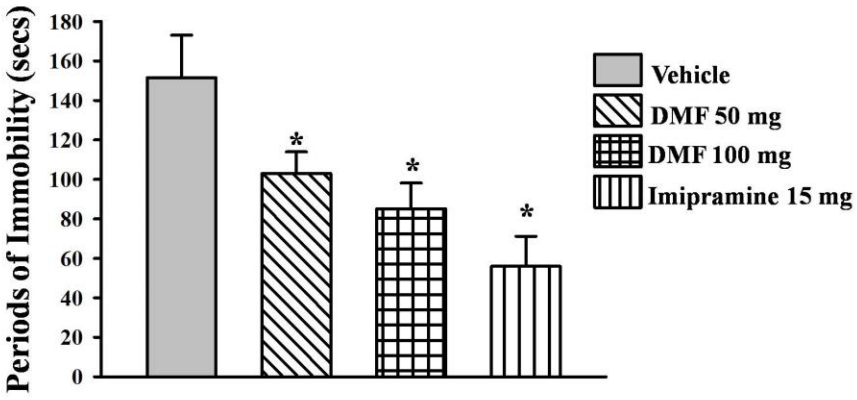

Fig 6: Statistical analysis of periods of immobility following chronic drug administration in the tail suspension test. Both doses of DMF reduced periods of immobility. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=8 per group

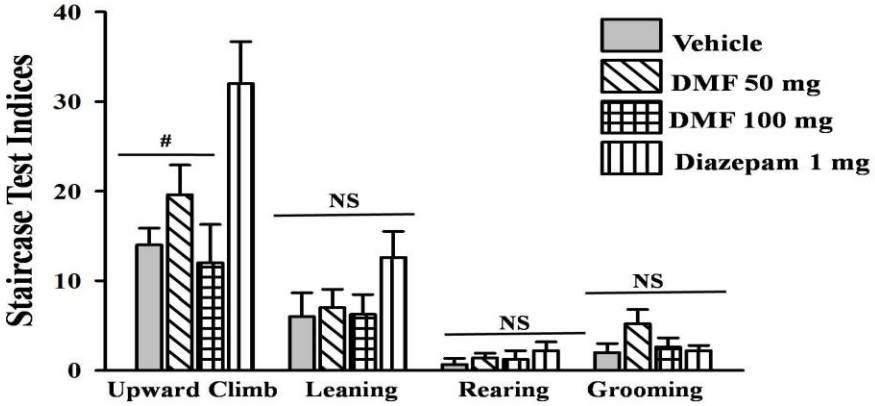

Fig 7: Statistical analysis of staircase test indices. Both doses of DMF did not increase number of upward climbs, or reduce leaning or episodes of rearing and grooming. Data is expressed as mean ± SEM. $^{\#}p<0.05$ compared to imipramine, NS, Not significant; n=7 per group

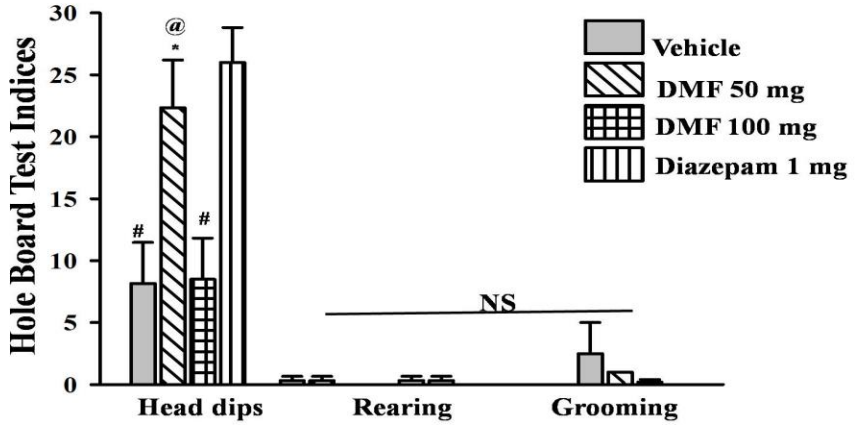

Fig 8: Statistical analysis of hole-board test indices. High dose DMF (100 mg) did not increase number of head dips or reduce episodes of rearing or grooming. Low dose (50 mg) improved number of head dips, which was significantly different from the vehicle. Data is expressed as mean ± SEM. $^{*}p<0.05$ compared to vehicle, $^{\#}p<0.05$ compared to imipramine, $^{@}p<0.05$ compared to DMF 100 mg; NS, Not significant; n=7 per group

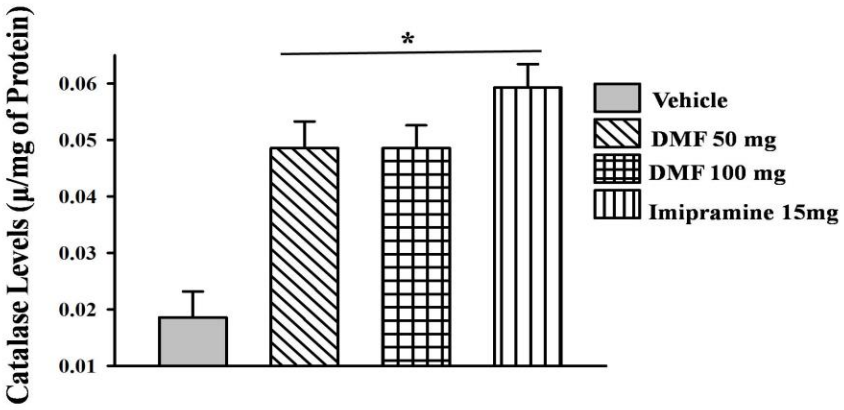

Fig 9: Statistical analysis of levels of brain catalase post chronic drug administration. Both doses of DMF increased brain catalase levels. Data is expressed as mean ± SEM. $^{*}p<0.05$ compared to vehicle; n=8 per group

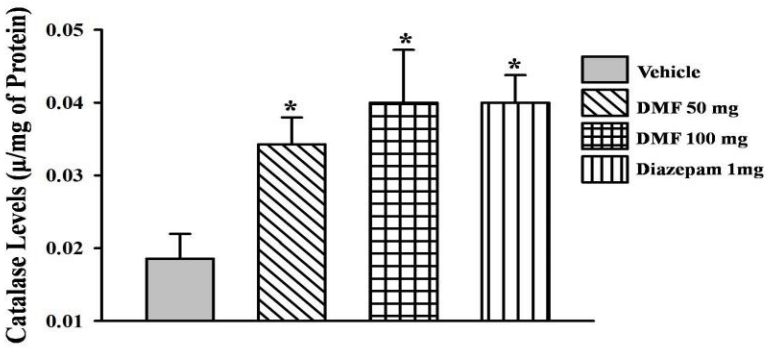

Fig 10: Statistical analysis of levels of brain catalase post chronic drug administration. Both doses of DMF increased brain catalase levels. Data is expressed as mean ± SEM. *p<0.05 compared to vehicle; n=7 per group

The tail suspension test has become one of the most widely used models for assessing antidepressant-like activity in mice. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tails, will develop an immobile posture. Immobility is defined as the absence of initiated movements and includes passive swaying. Mice exhibit similar periods of immobility in TST as in FST based on the treatment they have received prior to the test [23]. Both doses of DMF reduced periods of immobility in both forced swim and tail suspension tests following acute and chronic drug treatment indicative of antidepressant activity.

The staircase test for evaluating anxiolysis as described by Thiebot *et al.* [24] and modified by Simiand *et al.,* [19] measures the conflict between anxiety and exploratory behaviour when rodents are placed in a new environment. In the staircase, the numbers of steps climbed and episodes of rearing are measured as behavioural parameters of exploratory/locomotor activity and anxiety, respectively [19,25]. An increase in the number of upward climb indicates absence of anxiety. Also, increase in periods of rearing, grooming and lean on the wall indicates the presence of anxiety in this animal model. DMF did not increase number of upward climb or reduce episodes of rearing and grooming suggestive of absence of anxiolytic activity probably at doses used in this study.

The hole-board consists of a square arena measuring 3 cm with 16 holes equidistant from one another that rodents can explore by poking their heads. The head dipping is a validated measure of exploratory activity and anxiety in rodents. The number of head-dips is assumed to be inversely proportional to the anxiety state. In addition, increase in the periods of rearing and grooming implies anxiety in the animal model [20,26,27]. In the acute phase of the study, low dose DMF increased number of head dips in the hole-board, though not significantly different from the vehicle treated animals, however, low dose DMF significantly improved number of head dips in the hole-board test post chronic drug treatment.

Oxidative stress is particularly facilitated in the brain due to the high oxygen utilization, generation of free radicals, insufficient antioxidant defence mechanisms, high lipid content and excitotoxicity [28]. Oxidative stress plays significant role in pathophysiology of major depression via actions of free radicals, non-radical molecules, and reactive oxygen and nitrogen species. The brain is thus notably susceptible to the damages caused by free radicals. Cytosolic-enzyme catalase is a component of the antioxidant defense system that reduces hydrogen peroxide to water. An increase in free radical formation is accompanied by an immediate compensatory increase in catalase activity, which may be a long-term

compensatory mechanism. Products of oxidative stress represent important parameters for measuring and predicting depression status as well as for determining effectiveness of administrated antidepressants [28-30]. Stress has been shown to increase pro-oxidants production and an imbalance between superoxide dismutase and catalase activities. Antioxidant enzymes may thus be markers of major depression as catalase levels were found to be decreased in depressed patients but returned to normal levels after treatment with antidepressants [11,30]. Consistent with the reduction in periods of immobility in the forced swim and tail suspension tests, DMF improved levels of brain catalase indicative of reduced oxidative stress. This could be a possible mechanism of antidepressant activity of DMF, though more mechanistic studies will be needed to confirm these findings.

The anxiety levels in mice associated with the oxidative status in both neuronal and glial cells in the cerebellum and hippocampus, in cortical neurons and in peripheral leucocytes (monocytes, granulocytes and lymphocytes), reveals increased levels of reactive oxygen species in the brain and in the peripheral system of anxious mice [31]. There is a direct correlation between oxidative stress and anxiety. Studies have shown that anxiolytic agents decrease reactive oxygen species and reactive nitrogen species through scavenging radicals and suppression of the oxidative stress pathway, which further protect against oxidative stress-induced neuronal damage and may result in the remission and functional recovery of anxiety symptoms [13,28,31]. Contrary to some of the results from neurobehavioural evaluation in the staircase and hole-board tests, treatment with DMF improved levels of brain catalase post chronic treatment at both doses. We speculate that low dose DMF might be more potent in producing anxiolysis as seen from the effects of DMF in the hole-board tests, again more studies will be carried out to confirm/refute this hypothesis.

Taken together, our results indicate antidepressant and anxiolytic potential of DMF in mice via elevation of brain catalase levels.

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