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Original Research Article

Evaluation of the anti-depressant potential of metformin in conditioned defeat model in golden Syrian hamsters

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ABSTRACT

Background: Depression is a prevalent mood condition that has an impact on daily functioning. Globally, depression affects 264 million people. The current pharmacotherapy of depression has a lot of shortcomings. Therefore, there is a need to explore newer therapy that alleviate the symptoms of depression. Metformin was found to possess antioxidant potential and hypothesized to decrease the levels of branched-chain amino-acids essential for tryptophan uptake (precursor for serotonin synthesis). The study was designed to validate the efficacy of metformin as an anti-depressant in conditioned defeat model in male golden Syrian hamsters using open field test (OFT), forced swim test (FST) and Serum serotonin levels.
Methods: After obtaining IAEC approval, the study was carried out using 8 golden Syrian hamsters each that were randomly assigned to four groups. The disease control group received 1mL normal saline, positive control was given fluoxetine 12 mg/kg, two groups of metformin 240 mg/kg given pre-insult and post-insult. The variables assessed on every third day included OFT and FST. Following the behavioral tests, serotonin-ELISA was done. To analyse the outcomes, appropriate statistical tests were applied.
Results: On standardization, the model was established to a 16-day model. Further, results highlighted a significant difference in OFT, FST and serotonin levels with the metformin group and fluoxetine compared to disease control ($p<0.001$). However, no significant difference was observed between the fluoxetine and metformin groups ($p>0.05$), signifying the comparable results.
Conclusions: Metformin (240 mg/kg) alleviated the depressive symptoms by modulating both behavioral and serotonin levels.

Keywords: Forced swim test, Serotonin, Open field test

INTRODUCTION

Depression, often known as major depressive disorder (MDD), is a mental condition that is extremely common but also has the potential to be fatal. Changes in appetite or weight, irregular sleep patterns, psychomotor agitation or retardation, exhaustion, a loss of mental clarity or concentration, feelings of worthlessness or excessive guilt, and suicidal thoughts are among the secondary symptoms. The patient may experience one symptom or all of them.[1]

Depression has emerged as a pandemic claiming a malignant toll on health affecting more than 264 million people globally.[2] Clinical and etiological heterogeneity of the disease has made it an arduous task to elucidate the exact pathophysiological mechanism. One of most studied hypothesis is the depletion of monoamines like serotonin (5-HT), nor-epinephrine (NE) and dopamine (DA), extensively targeted by pharmaceutical industries to manufacture drugs that alleviate depressive symptoms. Other theories put-forward include changes in the hypothalamus-pituitary–adrenal axis, inflammation in the

brain or any structural of functional alterations leading to cognitive and behavioural changes.[3]

Numerous antidepressant medications, such as monoamine oxidase inhibitors, tri-cyclic antidepressants, selective serotonin re-uptake inhibitors, and serotonin non-epinephrine re-uptake inhibitors, have currently received FDA approval. However, even with the highest compliance to pharmacotherapy, the remission rates remain a meagre 30-40%.[4] The alarming public health concern, very little insight on the etiopathogenesis, heterogeneity of depression, pitfalls in the current pharmacotherapy has alerted the researchers in search of a better alternative.

Metformin is a biguanide class of oral anti-diabetic drug used as a first-line medication in type 2-diabetes mellitus.[5] We sought to explore the anti-depressant potential of metformin based on a hypothesis which states that chronic exposure to stress induces various biochemical, endocrine and immune changes than acute stress. Rise in the reactive oxygen species (ROS) cause oxidative damage in the brain. Additionally, this inhibits neurogenesis, worsens synaptic and neural plasticity, and causes neurodegeneration and mitochondrial malfunction. Thus, depression sets in.[6] Another theory focused on the role of amino acid in depression. Free fatty acids (FFA), branched chain amino acids (BCAA), and insulin can all have an impact on tryptophan availability in the brain. Tryptophan absorption into the brain is competitively inhibited by these BCAAs. Because tryptophan is an important amino acid and a precursor to serotonin, a lack of it can lead to lower levels of serotonin and cause depression.[7,8] Modulating the amount of BCAA can aid as a completely unique target of anti-depressant drugs. Zemdegs et al research demonstrates that metformin has the distinct ability to lower levels of circulating BCAA.[9]

Due to their innate territorial hostility, Syrian hamsters have the advantage over other rodent species like rats and mice. In ritualised agonistic bouts, hostility is inherent, and severity of the encounters is typically lower. As a result, most of the stress that is caused is psychological.[10] Conditional and social defeat stress is an ethologically relevant model to specifically instigate stress, anxiety and depression symptoms with altered neurobiological mechanisms where male golden syrian (GS) hamsters (Mesocricetus auratus) display evident changes in social behaviour following at least one exposure of social interaction, increasing the validity of the experimental model.[11] Female hamsters were excluded as previous studies indicated that very little aggression was displayed while in oestrous.[12]

With the dearth of studies exploring the antidepressant potential of metformin, we aimed to evaluate the effect of metformin and fluoxetine in conditioned defeat model in GS hamster using OFT and FST. The biochemical assessment of serum serotonin levels was done using ELISA.

METHODS

Animal ethics committee permission

The institutional animal ethics committee permission was sought before the commencement of the study (IAEC/GSMC/07/2019). Animals randomly bred in the centre for animal studies of the Tata Memorial Centre-Advanced Centre for Treatment, research and Education in Cancer (ACTREC), Kharghar, Mumbai, were used. The study was conducted in accordance with the CCSEA guidelines between February 2020 to September 2020.

Experimental animals

In our investigation, 54 male GS hamsters were used, with the smaller (submissive) hamsters weighing between 80 and 180 gm and the larger (dominant) hamsters weighing over 180 gm. All of the hamsters were 8 to 9 weeks old.

Husbandry condition

Animals were maintained and acclimatized in the centre for animal study of Seth GSMC, Mumbai in conditions par with the CCSEA guidelines. Animals were transported from ACTREC with care; a suitable air-conditioned truck was employed, and each animal was housed in a separate cage and quarantined for two weeks in our facility.

Study drugs

Study involved Metformin- 240 mg/kg PO (per oral) and fluoxetine-12 mg/kg PO. The study drugs were procured from Sigma Aldrich, Mumbai, in pure powder form as an actual pharmacological ingredient. The doses were based on the conversions of doses used in previous studies in rats.[6] Then the doses were extrapolated using suitable formulae.[13] Serum serotonin ELISA kits for hamsters were purchased from KrishGen Biosystems employing sandwich ELISA.

Study procedure

We carried out the standardisation phase, where the exact number of days the model would be performed would be established, with an aim to establish the model at our institute. After a period of quarantine, all the hamsters were handled daily for 10 minutes by gently picking up the animal by its scruff and keeping it back in the cage so that handling does not act as a confounding factor. Four larger hamsters were utilised for the model's induction during the standardisation phase, which included two groups with eight animals each: a disease control group and a normal control group. Flow of procedure followed in conditioned defeat model has been depicted in Figure 1.[14-17]

The smaller (loser) hamsters were evaluated for behaviour after the experiment on day 0 and every third day using the OFT, noting how much time was spent in the central zone and how many times the central line was crossed using

Mazemaster 2.0 software.[18] FST was performed to evaluate the total duration of immobility in 5 minutes (training given the day before).[19] After the standardisation, phase-II of experiment was carried out to test for efficacy of the drugs by treating the animals with study drugs orally at selected doses for the number of days established in standardization phase. The details of the animal distribution are tabulated in Table 1. Figure 2 describes the time-line followed in phase-II. In addition to behaviour tests, serum serotonin assay was performed under aseptic precautions using retro-orbital blood collection.

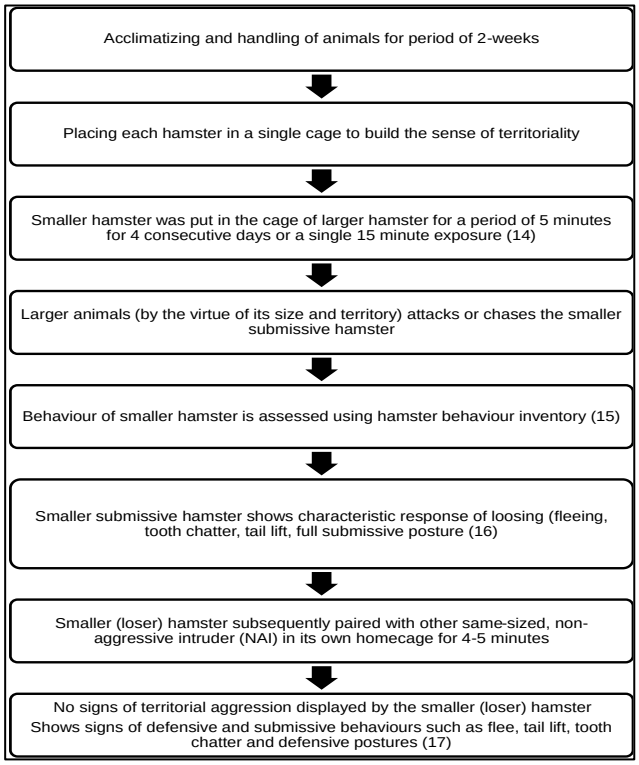

Figure 1: Conditioned defeat model.

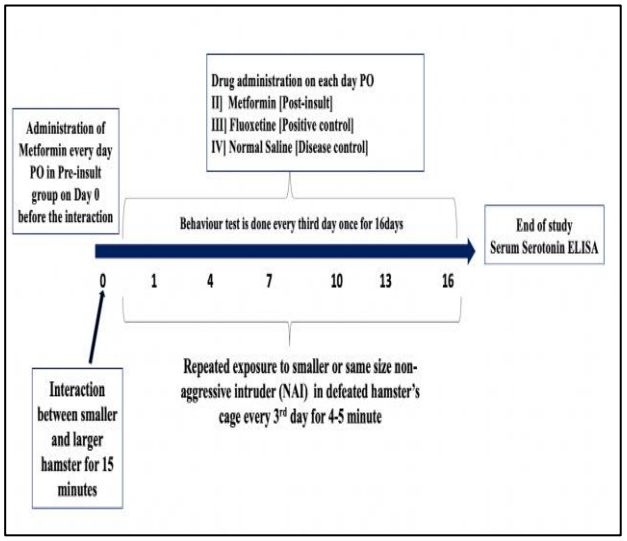

Figure 2: Timeline of phase-II.

Statistical analysis

Data was analysed using GraphPad Instat software version no 3.06. Level of significance was set at $p<0.05$. Normality was tested by Shapiro-Wilk test. In the standardization phase, to establish the model, comparison between normal control and disease control was made using unpaired t test. Determination of the number of days for the conditioned defeat model to be established, we utilised repeated measures ANOVA. Parametric data was analysed using one-way ANOVA. Relevant post-hoc test like Tukey's test was followed.

RESULTS

Results of standardization

Standardization was carried out with the aim of standardising the model and determining an exact number of days to undertake conditioned defeat model. Disease control and normal control were two groups (n=8) in the standardisation phase.

On OFT, there was a significant decrease in total time spent in central zone and total number of central line crossings in the disease control compared to normal control ($p<0.05$). Also, a significant increase in the total duration of immobility in the disease group was noted when compared to normal control with $p<0.05$. As a result, the OFT and FST data suggested that the conditioned defeat model was standardised as the hamster exhibited depressive symptoms (Figure 3).

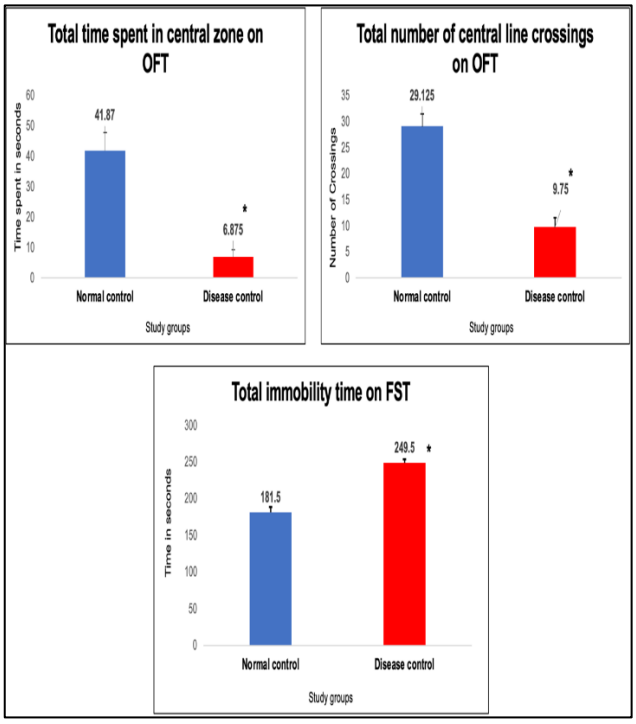

Figure 3: Results of standardization phase.
*P<0.05 vs normal control; using unpaired t test; OFT: Open field test; FST: Forced swim test.

The social interaction between the previously defeated hamster and the non-aggressive intruder (NAI) was scheduled to last until day 30 in order to standardise the number of days to carry out the conditioned defeat model. When the NAI was placed by day-19, it was seen that the previously defeated hamster's aggressive behaviours had reverted. The results have been tabulated in Table 2. All the values passed normality test. To compare results post-test, we used a repeated measure ANOVA and a post hoc Tuckey test. P<0.05 was obtained, considered as significant (Table 2). We observed that the there was a significant difference in the total time spent in central zone, total number of central lines crossings on OFT and immobility time on FST between day-1 and day-19 and day-22. However, no significant difference in these parameters between day-1 to 16. This suggests that the model was standardised to a 16-day model based on results of OFT and FST.

Results of phase-II

After the model was standardized to 16-days, we carried out phase-II where we compared the effect of metformin groups with fluoxetine in conditioned defeat model of depression with immobility time on FST and the time spent central zone and number of central line crossings on OFT. Serum serotonin ELISA was performed by employing sandwich ELISA technique. The results of phase-II have been depicted in Figure 4 and Table 3. There was a significant increase in the total time spent in central zone and number of central line crossings on OFT and statistically significant reduction in the immobility time on FST in the three study groups (Positive control and the pre-insult metformin group and post-insult metformin group) compared to disease control (p<0.001). However, the three study groups were comparable to each other.

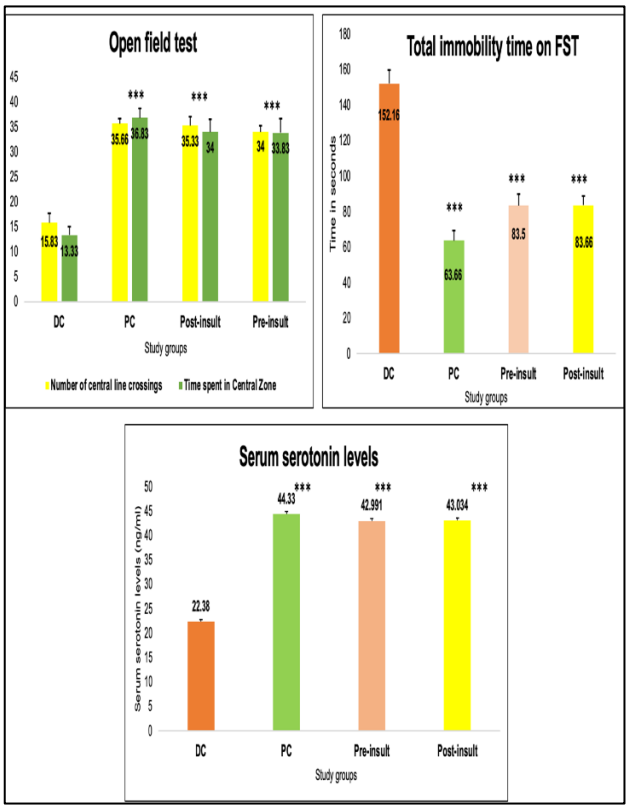

Figure 4: Results of phase-II of the test.

***P<0.001 vs. disease control group; ANOVA and post hoc Tukey's test; OFT: Open field test; FST: Forced swim test; PC-positive control and DC as disease control.

Table 1: Animal distribution in phase-II (PO: per oral) (n=8).

Groups	Drug	Dosage	Smaller hamster (n)	Dominant (n)
Disease control (DC)	Normal saline (NS)	1 ml PO	8	2
Positive control (PC)	Fluoxetine hydrochloride	12 mg/kg PO	8	2
Pre-insult treatment group	Metformin hydrochloride	240 mg/kg PO	8	2
Post-insult treatment group	Metformin hydrochloride	240 mg/kg PO	8	2

Table 2: Results of standardization phase to establish the number of days.

Days	Time spent in central zone on OFT in seconds (Mean±SD)	Number of central line crossings on OFT (Mean±SD)	Immobility time in seconds on FST (Mean±SD)
1	6.875±2.47	9.75±1.83	249.5±4.53
4	7±2.92	9.65±1.06	251.25±8.77
7	7.625±2.97	10.12±1.72	247.5±10.51
10	8.125±1.55	10.375±1.30	245.25±7.77
13	10.625±2.32	10.125±1.72	240.12±5.51
16	10.75±2.81	11.125±1.45	240.125±3.62
19	28.625±3.88*	18.875±2.74*	198±6.16*
22	41.875±5.84*	29.125±2.35*	181.5±7.59*

*P<0.05 when compared to day-1 on repeated measures ANOVA and post-hoc Tukey's test; SD: Standard deviation; OFT: Open field test; FST: Forced swim test.

Table 3: Results of phase-II.

Groups (n=8/ group)	Time spent in central zone on OFT. Mean±SD (seconds)	Number of central line crossings on OFT. Mean±SD (number)	Total immobility time on FST, mean±SD (seconds)	Serum serotonin levels, mean±SD (ng/ml)
Disease control	15.83±1.93	13.33±1.75	152.16±7.54	22.38±0.43
Pre-insult metformin	34±1.26***	33.83±2.85***	83.5±6.28***	42.991±0.43***
Post-insult metformin	35.33±1.75***	34±2.6***	83.66±5.31***	43.034±0.54***
Positive control	35.66±1.03***	36.83±1.83***	63.66±5.33***	44.33±0.58***

***P<0.001 vs. disease control group; ANOVA and post hoc Tukey's test; SD: Standard deviation; OFT: Open field test; FST: Forced swim test.

DISCUSSION

In our study, standardization was carried out and established that the conditioned defeat model worked till day-16 of the study period. The model showed highly significant ($p<0.05$) reduction in total time spent in the central zone and number of central line crossing on OFT and FST revealed similar results with significant increase in the total immobility time. In Phase-II of the study, the metformin treated groups showed a significant ($p<0.001$) antidepressant effect when compared to disease control (normal saline) on OFT and FST in GS hamsters. The results confirmed that the reduction in duration of immobility on FST with metformin treated groups was due to their antidepressant effect. The metformin treated groups showed a significant ($p<0.001$) antidepressant effect when compared to disease control (normal saline) in the serum serotonin levels. The serum serotonin levels were also significantly increased which proves that Metformin has an anti-depressant effect. After further analysis, the results confirmed that there was no statistical difference in metformin treated groups when compared to the fluoxetine (positive control) with respect to OFT, FST and serum serotonin ELISA.

In a study using adult C57BL/6 male mice exposed to chronic unpredictable to stress for 6 weeks to induce depression. It comprised of four study groups: vehicle controlled, fluoxetine [positive control], metformin, or a combination of fluoxetine and metformin. The results show that, the combination of fluoxetine and metformin treatment was found to be more effective approach than fluoxetine alone in a short term by increasing IGF2 levels in the dorsal hippocampus. In this study they have evaluated the combined effect of metformin and fluoxetine as an anti-depressant drug in a mouse model of depression. Whereas in our study, we tried to evaluate the efficacy of metformin alone as an anti-depressant drug.[20]

A study on mice which used bacterial endotoxin-lipopolysaccharide (LPS) to induce depressive like behavior exhibited increased immobility on both FST and tail-suspension test (TST) with an increase in miniature excitatory post-synaptic current (mEPSC). In Metformin treated LPS mice, there was a decrease in the immobility time in FST and TST. Also, presynaptic glutaminergic release was significantly increased in LPS-induced mice when compared to metformin treated group. These results show the evidence that metformin ameliorates depressive-like behaviors.[21]

In another study where high fat diet (HFD) was hypothesised to depression pathogenesis. The study aimed to evaluate the anti-depressant and cognitive potential of metformin in chronic restraint stress model in HFD rats and non-HFD rats. Authors observed a statistically significant improvement in the combination group of fluoxetine and metformin in depressive-like symptoms, with a better glucose and lipid control, increased adiponectin and brain-derived neurotrophic factor (BDNF) expression, and reduced corticosterone and hippocampal c-Jun repression in the HFD rat. Here, the combination proved to be preferable as an anti-depressant. Unlike, our study aimed to explore the potential benefit of metformin alone as an anti-depressant drug.[22]

Das et al conducted a study conducted using Wistar rats to evaluate the neuroprotective effect of metformin on a chronic FST. Behavioural parameters like time of fall in rotarod, locomotor activity in photo actometer, number of correct entries on radial maze, anti-oxidant effects of metformin via assessment of superoxide dismutase (SOD) level and the malondialdehyde (MAD) was carried out. Metformin showed neuroprotective effect via anti-oxidant effect, thus proving its role as a novel anti-depressant. In this study, FST was used an inescapable social stress. However, employment of conditioned defeat model in our study had a better face and construct validity as compared to chronic FST.[6]

The increase in central line crossing and duration spent in central zone on OFT and decrease in duration of immobility on FST due to metformin administration may be regarded as anti-depressant effect. Additionally, an increase in serum serotonin levels on ELISA may contribute to its anti-depressant effect.

Limitations

The anti-oxidant effect of metformin could have been assessed by estimating superoxide dismutase (SOD) as well as the malondialdehyde (MDA) levels. The combined effects of the fluoxetine as well as metformin were not assessed.

CONCLUSION

The experimental data concluded that the conditioned defeat model for a period of 16-days causes behavioural and biochemical changes with Metformin 240 mg/kg and showed an improvement in the depressive symptoms in male GS hamsters. The role of prophylactic Metformin in depression is inconclusive.

Recommendations

It is suggested that future studies explore the potential benefits of metformin as an anti-depressant on an array of pre-clinical models and also research the combination effect of fluoxetine and metformin in depression.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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