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Original Research Article

An experimental study to evaluate the role of aspirin and metformin in prevention of depression in rats

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ABSTRACT

Background: Depression was seen to be associated with an increased level of inflammatory biomarkers along with the disturbance in the monoamine neurotransmitter system. Current therapies are mostly focussed on the neurotransmitters imbalance but due to increasing cases of treatment failure there is a need to shift our treatment focus to other potential therapies. This study aimed to evaluate the preventive role of aspirin and metformin in stress induced model of depression in wistar rats.

Methods: Fifty four wistar rats were randomly divided into nine groups as normal control, experimental control, aspirin (30 mg/kg, 60 mg/kg), metformin (50 mg/kg, 100 mg/kg), two combination groups and imipramine (15 mg/kg). Depression model was created by the induction of chronic unpredictable mild stress (CUMS) for consecutive 28 days. Behavioural assessment was done by evaluating immobility time in forced swim test (FST) and sucrose preference ratio (SPR) in sucrose preference test. The data were analysed by analysis of variance (ANOVA) test using SPSS software. $P < 0.05$ was considered to be statistically significant.

Results: The CUMS led to an increase in immobility time and decrease in SPR. Aspirin and Metformin alone and their combinations showed statistically significant response in preventing the immobility time to increase ($p < 0.001$) and SPR to decrease ($p < 0.001$). However the response of Aspirin was comparable with Imipramine but the response of Metformin was not as significant as of Imipramine ($p > 0.05$).

Conclusions: Aspirin and metformin might have a potential role in the prevention of depression.

Keywords: Aspirin, Chronic unpredictable mild stress, Depression, Inflammation, Metformin

INTRODUCTION

Major depressive disorder (MDD) is an extremely prevalent neuropsychiatric disease that affects around twenty five to thirty percent of the patients visiting outpatient departments.¹ Besides the disturbance in monoamine neurotransmitter system, the alteration in hypothalamic-pituitary-adrenal axis (HPA) is also an important contributing factor to the pathology of depression.² Increased activity of HPA axis leads to an increase in cortisol level which along with dysfunction of the central serotonergic system has a consequential effect on pathology of depression.³ Several meta-analyses have

gathered the colossal evidence, where pro-inflammatory markers like C reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)- α have been found at increased levels among patients with depression which indicates that the immune system is closely associated with depression.^{4,5}

Inflammatory markers like IL-1 β regulates the expression of the serotonin transporter gene and IL-6 may also contribute to the inhibition of proliferation.^{6,7} This may be consistent with the hypothesis that hippocampal neurogenesis is necessary for the treatment of depression. It was also postulated that IL-6 can be utilised as a new

target for the therapy of depression.⁸ TNF- α could activate HPA axis and directly activate indoleamine-2,3-dioxygenase (IDO), which is expressed in macrophages and dendritic cells in the brain and then through kynurenine pathway catabolize tryptophan which is the substrate for serotonin synthesis.^{9,10}

Considering the role of inflammatory pathology in depression, this study was designed to look for the preventive role of anti-inflammatory drugs in stress induced model of depression in Wistar rats. The drugs used in this study were aspirin and metformin.

Aspirin cause irreversible inhibition of cyclooxygenase enzyme by covalently modifying COX-1 and COX-2 activity.¹¹ Aspirin had been studied and in use for a wide variety of disorders like prevention of thrombus formation in patients of myocardial infarction and stroke, it is also used in the prevention of wide variety of cancers like colon cancer.¹² According to the study, COX-1 and COX-2 are involved in neuroinflammation for leucocyte recruitment and reduction in COX-1 activity is beneficial in inflammation reduction. According to an experiment conducted by Choi et al, intracerebroventricular LPS induced neuroinflammation was attenuated in COX-1 mice as compared to COX-2 mice. They also conducted another study to show the response of COX-1 gene deletion on neuroinflammation.¹³

Metformin is a drug classified under the biguanide family of anti-diabetic medications and is primarily used to treat hyperglycaemia and insulin resistance in type 2 diabetic patients and can be safely given for long term.¹⁴

Besides its use in diabetes Metformin has lots of other pleiotropic effects like it was seen beneficial in cardiovascular diseases and various types of cancers with very few adverse effects.¹⁵ It was also been demonstrated that metformin could inhibit nuclear factor- κ B (NF- κ B) signalling. NF- κ B is involved in the pathophysiology of various diseases like diabetes mellitus (DM) and

cardiovascular disease (CVD).¹⁶ By making NF- κ B as one of its targets it could be helpful in all those inflammatory pathologies in which is involved as a contributing factor.¹⁷ So we hypothesised that because of its anti-inflammatory potential of aspirin and metformin they might have some effect in the prevention of Depression.

METHODS

After being approved from institutional animal ethics committee (97/IAEC/2018 dated- 20/8/2018) study was carried out in 54 healthy adult albino wistar rats of either sex, who were not different in their age and body weight (150-200gm) and were kept under standard laboratory conditions of temperature and humidity (25 \pm 2°C, 70%). They were provided pellet food and water ad libitum with 12 hours light/dark cycle except on days of stress. Rats were bought to the laboratory one week before starting the experiment. After one week of acclimatisation rats were randomly divided into 9 groups of 6 rats in each group as group I was normal control, group II was stress control, group III was treated with aspirin (30 mg/kg), group IV was treated with aspirin (60 mg/kg), group V was metformin (50 mg/kg), group VI was metformin (100 mg/kg), group VII was treated aspirin (30 mg/kg) with metformin (50 mg/kg) combination, group VIII was treated with combination of aspirin (60 mg/kg) and metformin (100 mg/kg), group IX was imipramine (15 mg/kg) treated group. All the drugs were given per orally by dissolving in distilled water. During the entire course of the study, the standard protocols were followed. Powdered forms of aspirin and metformin were purchased from HIMEDIA, sucrose powder was purchased from Fischer chemicals and imipramine powder was purchased from TCI chemicals.

Model of depression was created by the induction of hronic unpredictable mild stress (CUMS). The CUMS procedure is the variation of methods as described in the previous literature.¹⁸

Table 1: Schedule of CUMS for 28 days.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
W1	Tail pinch 5 min	Confined space 90 min	Water deprivation 24 hours	Tilted cage 90 min	Confined space 90 min	Tail pinch 5 min	Food deprivation 24 hours
W2	Confined space 90 min	Tilted cage 90 min	Tail pinch 5min	Water deprivation 24 hours	Tilted cage 90 min	Confined space 90 min	Food deprivation 24 hours
W3	Tail pinch 5 min	Confined space 90 min	Food deprivation 24 hours	Tilted cage 90 min	Water deprivation 24 hours	Tilted cage 90 min	Tail pinch 5min
W4	Water deprivation 24 hours	Tilted cage 90 min	Confined space 90 min	Tail pinch 5 min	Confined space 90 min	Tilted cage 90 min	Food deprivation 24 hours

CUMS- chronic unpredictable mild stress; W1- week 1, W2- week 2, W3- week 3, W4- week 4.

The stressors for CUMS were applied each day for consecutive 28 days in all the groups except the normal control. The stressors include tail pinch for 5 min, confined space for 90 min, Water deprivation for 24 hours, food deprivation for 24 hours, tilted cage at 45 degrees for 90 min. The stressors were applied with the pre decided schedule as given in Table 1. The stress schedule was changed every week to avoid habituation of the rats to the stress.¹⁹ Respective drugs were given along with CUMS for 4 weeks every morning at a fixed time to the treatment groups and distilled water at the same time to control groups.

Behavioural tests

Forced swim test: Forced swim test (FST) was done in the transparent cylinder of height 40 cm and diameter of 20 cm. It was filled with water up to the depth of 30 cm and then the rats were placed in the cylinder. Initially the rats showed excessive activity and tried to escape. After some time they reduce their activity and eventually became “immobile”. Rat was considered to be immobile when they did only that much movements that were essential to keep its head above water. At day 0 only habituation session of the rats were done in the FST apparatus to make them familiar with the process. The test session was started after 24 hours on day 1.

On day 1, FST test session was performed for 5 min and the duration of immobility was recorded in all the groups. FST test session was performed again on day 2, after 1 hour of giving the respective treatment to each group. Then final assessment was done at day 28. The water was changed after each animal testing. The changes in immobility duration were recorded on day 1, day 2 and day 28.

Sucrose preference test: Anhedonia in rats can also be measured by another behavioural test that is sucrose preference test. At day 1 after a 12-hour period of food and water deprivation, rats were given free access to either of two bottles containing 1% sucrose solution or water provided in their cages. The positions of the two bottles were switched after 30 min to avoid position preference. After 1 hour, the volumes of consumed

sucrose solution and water were recorded. The changes in sucrose preference ratio were recorded on day 1 and day 28. The sucrose preference ratio (SPR) were calculated according to the following equation:²⁰

$$SPR = \frac{\text{Sucrose intake (ml)}}{\text{Sucrose intake (ml)} + \text{Water intake (ml)}}$$

Data were summarised as mean±SD. Intergroup comparisons of immobility duration in FST and SPR were done using analysis of variance (ANOVA) followed by post hoc Tukey’s Honestly significant difference (HSD) test. A p value of <0.05 was considered statistically significant.

RESULTS

Assessment of anti-depressant activity using forced swim test

Inter group comparison was done using ANOVA test at day 1, day 2 and day 28 as shown in Table 2. On day 1, mean duration of immobility ranged from 62.17±5.00 sec to 66.67±3.72 sec. Statistically, the intergroup differences were not significant (p=0.456). On day 2, mean duration of immobility ranged from 64.33±1.37 sec to 73.50±10.31 sec. Statistically, there was no significant intergroup difference in mean immobility times (p=0.256). While on day 28, mean duration of immobility ranged from 61.83±2.32 sec to 136.17±4.96 sec. Statistically, the intergroup differences were significant (p<0.001).

Assessment of anti-depressant activity using sucrose preference ratio

Inter group comparison of sucrose preference ratio before inducing CUMS at day1 and after inducing CUMS at day 28 was done using ANOVA as shown in table 3. Before induction of CUMS at Day 1 mean SPR values ranged from 0.900±0.026 to 0.920±0.033, however the intergroup differences as assessed by ANOVA were not found to be significant (p=0.989). After induction of CUMS mean SPR values ranged from 0.523±0.077 to 0.908±0.022. Statistically, the intergroup differences were significant (p<0.001).

Table 2: Intergroup comparisons of duration of immobility in FST using ANOVA at day 1, day 2 and day 28.

Groups	Day 1		Day 2		Day 28	
	Mean	SD	Mean	SD	Mean	SD
I	66.67	3.72	67.83	6.08	66.67	6.89
II	64.67	3.98	68.67	4.89	136.17	4.96
III	66.67	4.32	69.17	2.40	70.17	5.38
IV	63.67	4.63	64.33	1.37	70.33	3.20
V	63.17	3.49	67.33	4.37	105.67	9.16
VI	62.17	5.00	66.50	3.15	96.83	7.11
VII	66.00	5.22	67.00	3.03	69.17	2.48
VIII	66.33	3.93	73.50	10.31	71.00	9.03
IX	66.83	4.40	67.50	6.25	61.83	2.32
Statistical significance (ANOVA)	F=0.991; p=0.456		F=1.325; p=0.256		F=97.755; p<0.001	

SD- standard deviation; F- F statistic value of ANOVA; *- p-value <0.05=statistically significant

Table 3: Intergroup comparisons of duration of sucrose preference ratio in SPT using ANOVA at day 1 and day 28.

Group	Before		After	
	Mean	SD	Mean	SD
I	0.905	0.027	0.907	0.022
II	0.902	0.052	0.523	0.077
III	0.913	0.048	0.883	0.026
IV	0.912	0.023	0.882	0.023
V	0.905	0.030	0.655	0.143
VI	0.913	0.033	0.687	0.043
VII	0.900	0.026	0.872	0.022
VIII	0.920	0.033	0.900	0.015
IX	0.912	0.036	0.908	0.022
Statistical significance (ANOVA)	F=0.203; p=0.989		F=35.07; p<0.001	

SD- standard deviation; F- F statistic value of ANOVA; *- p value<0.05= statistically significant

Tukey post hoc analysis showed there was a significant difference between the groups at day 28 ($p<0.001$). The mean duration of immobility and SPR in standard group IX (imipramine-15 mg/kg) when compared by Tukey post hoc analysis to experimental control Group II at day 28 a significant difference was found between the two groups ($p<0.001$). But there was no significant difference between the normal control Group I and Group IX ($p=0.905$).

The mean duration of immobility and SPR in all the test groups when compared by Tukey post hoc analysis to stress control group II at day 28 a significant difference was found ($p<0.001$). When compared to normal control group I no significant difference was found with group III, group IV, group VII and group VIII ($p>0.05$). These groups when compared to standard group IX also they did not shown any significant difference ($p>0.05$). But the test group V and test group VI showed significant difference with both group I and group IX ($p<0.001$).

DISCUSSION

Keeping the inflammatory pathology of depression in mind this study was designed to look for the preventive role of Aspirin and metformin in chronic stress induced depression and comparison with the standard anti-depressant drug Imipramine.

As per our results at day 1 there was no significant difference in duration of immobility in FST and sucrose preference ratio in SPT, which shows that the findings of all the groups were comparable before the start of the study and on day 2 also no statistically significant difference was observed. Day 2 evaluation was done to look for any acute effects of the drugs but in this study, no acute effect of any drug was observed in any of the treatment groups. This result can be justified as most of the anti-depressant actions of the drug is by the modulation in brain neurotransmission which takes the

time of around 3 to 4 weeks. There is a time delay in the onset of response because the effect is due to long term neuronal adaptations rather than acute modulation of transporters and receptors.²¹

At day 28 mean duration of immobility in FST and sucrose preference ratio in SPT was highest in the stress control group II in which only CUMS was given without any drug treatment. This shows that induction of CUMS can increase the immobility duration in the forced swim test. This result was also supported by the previous work which showed an increase in immobility duration after induction of CUMS in forced swim test.²² The similar type of result has been found in earlier study to look for the effect of stressor on sucrose preference test.²³ It has been seen that rodents naturally have a preference for sweetened foods and drinks and when given a two-bottle free-choice regimen with access to both sucrose solution and regular water they show preference towards sucrose solution and this preference is lost when exposed to stress based models of depression like CUMS.²⁴

The mean duration of immobility and SPR in standard Group IX (imipramine 15 mg/kg when compared to stress control group II at day 28 a significant difference was found between the two groups. This result showed that imipramine does not allow the immobility to increase in the forced swim test and SPR to decrease in sucrose preference test. A similar type of study was also performed previously on imipramine and they also demonstrated the decrease in immobility duration in FST after treatment with imipramine.²⁵

At day 28 the mean duration of immobility and SPR in the test group III (aspirin 30 mg/kg) and the test group IV (aspirin 60 mg/kg) at day 28 was not significantly increased from day 1. The above data showed that aspirin has positive effects on the prevention of depression as it decreases the immobility duration in FST in comparison to control group. The findings of our study are also supported by the study performed by Bhatt et al to look for the effect of aspirin in depression using experimentally induced model of depression with dexamethasone. They also found the decrease in immobility duration in forced swim test in rats receiving aspirin.²²

Our results demonstrated that effect of metformin was produced at both the doses as it was statistically significant from group II but not as much as standard group IX as significant difference was found between the two groups. This result can be supported by the study conducted by Shivavedi et al who used metformin in the dose of 25 mg/kg showed a decrease in immobility time in the forced swim test.²⁶ Another study was also conducted which used metformin in the doses of 50, 75, 100, 150 mg/kg and found that metformin could prevent the methamphetamine induced behavioural changes more at high doses.²⁷

The significant results in combination group VII and group VIII was might be due to aspirin as metformin alone was not able to show the significant result as that of Imipramine. There was no significant difference between the combination groups and aspirin alone groups ($p>0.05$), this shows that there was no benefit of adding metformin with aspirin on immobility time in FST and sucrose preference in SPR test as per our findings. The possible mechanism for the above results is the anti-inflammatory role of aspirin and metformin in depression as described above. There are various studies to support these results for aspirin but there are very limited studies on the role of metformin in depression. However, details of the complete mechanism have not yet been explored.

CONCLUSION

The study demonstrated the possible role of aspirin and metformin in the prevention of depression. Aspirin at both the doses 30 mg/kg and 60 mg/kg showed positive and significant response nearly similar to that of imipramine a conventional tricyclic anti-depressant, while metformin at both the doses 50 mg/kg and 100 mg/kg showed improvement in comparison to control but it is not as efficacious as imipramine as appeared in our results. The response of the combination of aspirin and metformin was similar to aspirin alone or imipramine group. In the light of above evidence further experiments are required with more parameters like inflammatory markers and neurotransmitter levels to look for the preventive role of aspirin and metformin in depression.

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Conflict of interest: None declared

Ethical approval: Study was approved by institutional animal ethics committee, KGMU, Lucknow (Project no-97/IAEC/2018)

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