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EFFECT OF DRUGS MODULATING SEROTONERGIC SYSTEM ON THE ANTI-DEPRESSANT ACTION OF FLUOXETINE AND DESIPRAMINE IN ALBINO MICE

Dr. Subir Kumar[1], Dr. Biswadeep Banerjee[2], Dr. Akash Chandra[3], Dr. Tanmoy Gangopadhyay[4]*, Dr. Vijay Kumar[5], Dr. Manpreet Saini[6]

[1]Associate Professor, Department of Pharmacology, Phulo Jhano Medical College, Dumka.
[2]Medical Officer (Jharkhand gov), Mohanpur, Deoghar
[3]Assistant Professor, Department of Pharmacology, Shahid Nirmal Mahato Medical College, Dhanbad
[4*]Associate Professor, Department of Pharmacology, Medical College Kolkata
[5]Professor, Department of Community Medicine, Laxmi Chandravansi Medical College and Hospital, Palamu
[6]Associate Professor, Department of Biochemistry, Laxmi Chandravansi Medical College and Hospital, Palamu

***Corresponding author:** Tanmoy Gangopadhyay
*Associate Professor, Department of Pharmacology, Medical College Kolkata

Abstract:

Introduction:

Depression is a complex and prevalent psychiatric disorder that necessitates a thorough understanding of its underlying mechanisms to develop more effective treatment strategies. This study investigated the effect of drugs modulating the serotonergic system on the antidepressant action of fluoxetine and desipramine in albino mice.

Material & Methods:

The two widely used behavioural tests, the tail suspension test and the chronic mild stress test, were employed to evaluate the antidepressant potential of these drugs.Six albino mice were included in each experimental group.

Result & discussion:

The results of this study revealed a noteworthy finding regarding the impact of ondansetron, a serotonin antagonist, on the antidepressant capabilities of fluoxetine and desipramine. Contrary to expectations, it was observed that ondansetron did not impair the antidepressant effects of either fluoxetine or desipramine. Instead, a surprising potentiation of their antidepressant actions was noted in the presence of ondansetron.

Conclusion:

These findings suggest that the serotonergic system, often implicated in the pathophysiology of depression, may have a more intricate role in antidepressant response than previously understood. Further research is warranted to elucidate the mechanisms underlying this unexpected interaction between serotonergic modulators and established antidepressants. Such insights may contribute to the development of novel therapeutic approaches for the treatment of depression, ultimately improving the lives of individuals suffering from this debilitating condition.

Key words: Fluoxetine, desipramine, depression, tail suspension test, chronic mild stress test

Introduction:

Depression is a global mental health challenge, affecting millions of individuals across the world.[1] Its complex aetiology has prompted extensive research into the neurochemical mechanisms underlying this debilitating condition.[2] Despite its prevalence, depression remains a complex and enigmatic disorder, with factors such as genetics, environment, and neurotransmitter systems intricately intertwined in its manifestation.[3]

Among the various neurotransmitter systems implicated in depression, the serotonergic system has garnered significant attention. Serotonin, often referred to as the "feel-good" neurotransmitter, plays a pivotal role in regulating mood and emotional well-being.[4] Dysregulation within this system has been linked to depressive symptoms and has thus become a focal point for therapeutic interventions.[5]In the quest to develop more effective antidepressant therapies, researchers have explored numerous avenues. Two widely studied antidepressants, fluoxetine and desipramine, have distinct mechanisms of action.[6] Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), focuses on enhancing serotonin availability, while desipramine, a tricyclic antidepressant, primarily influences norepinephrine reuptake.[7]

Additionally, the serotonin antagonist, ondansetron, has recently emerged as a potential player in the intricate landscape of depression treatment.[8] Ondansetron's unique role as a serotonin antagonist has sparked interest in its interaction with traditional antidepressants. Researchers are exploring whether combining ondansetron with established antidepressants could offer novel pathways to enhance treatment efficacy and provide relief to individuals battling depression.[9]

However, recent investigations have also delved into the intriguing interplay between these drugs and the serotonergic system, illuminating the potential for serotonergic synergy in alleviating depressive symptoms.[10]Albino mice, owing to their genetic homogeneity and suitability as experimental models, have become invaluable subjects in understanding the interactions between drugs that modulate the serotonergic system and traditional antidepressants.[11] By examining the effects of these compounds in albino mice, researchers seek to elucidate whether serotonergic modulation can augment the antidepressant actions of fluoxetine, desipramine, and ondansetron, paving the way for more efficacious treatment strategies.[12]

In this exploration of "Serotonergic Synergy," we embark on a journey through the intricate web of neurotransmitter interactions.[13] We delve into the intricate experiments conducted on albino mice, shedding light on how these tiny subjects may hold the key to enhancing the therapeutic potential of established antidepressants, both alone and in combination with ondansetron.[14,15] This investigation seeks to not only expand our understanding of the serotonergic system's role in depression but also offer potential avenues for the development of more potent and precise antidepressant interventions.

Material and methods:

The study was conducted at the Department of Pharmacology, VMMC & Safdarjung Hospital, New Delhi. Albino mice, aged 10-12 weeks and weighing 20-30 grams, were brought to our laboratory and housed in conditions of a 12-hour light/dark cycle, 65% humidity, and an ambient temperature of approximately 25°C. They had free access to both food and water. After a one-week acclimatization period, these animals were used for the experiment. Ethical approval for all experimental procedures was obtained from the Institutional Animals Ethics Committee of VMMC & SJH under approval number VMMC/IAEC/13, dated 04/03/2013.The drugs used in the study included Ondansetron (Sigma-Aldrich), Fluoxetine (Sigma-Aldrich), and Desipramine (Sigma-Aldrich). A 1% sucrose solution and Normal Saline (NS), which is a 0.9% NaCl solution in distilled water, were procured from the Drug Store Department of VMMC & SJH, New Delhi. All drugs were freshly prepared by dissolving their pure powder forms in distilled water.

Table 1: Treatment design of various groups

Drug	Dosage	No. of animals
Control	0.5 ml of normal saline;i.p.	6
Fluoxetine	10mg/kg; i.p.	6
Desipramine	15mg/kg; i.p.	6
Ondansetron	0.1 mg/kg; i.p.	6
Fluoxetine+Ondansetron	10mg/kg+0.1mg/kg;i.p.	6
Desipramine+Ondansetron	15mg/kg+0.1mg/kg;i.p.	6

***** In TST the various drugs and their combinations are given for 21 days and on the 22nd day the immobility time is measured.) (**In CST, the various drugs and their combinations are administered for 21 days, i.e., from day21 to day42 and the sucrose consumption is measured weekly, i.e., on day28, 35,and 42.) The doses of all the drugs were selected on the basis of previous studies from the literature.

Each group underwent two depression models: the Tail Suspension Test (TST) and the Chronic Mild Stress Test (CMS).

a. Tail Suspension Test (TST): In this model, we measured the total duration of immobility induced by suspending the mice by their tails.[16] The mice were isolated both acoustically and visually and suspended 50 cm above the floor using adhesive tape, positioned approximately 1 cm from the tip of the tail. We recorded the time of immobility during a 6-minute period, defining immobility as complete motionlessness. We administered the drugs and their combinations to the animals for 21 days, after which we conducted the TST on the 22nd day.

b. Chronic Mild Stress Test (CMS): In this procedure, animals were initially trained to consume a 1% sucrose solution.[17] The training involved presenting sucrose in their home cages following 14 hours of food and water deprivation. We measured sucrose intake by weighing pre-weighed bottles containing the sucrose solution at the end of the test. Subsequently, sucrose consumption was monitored weekly throughout the experiment under similar conditions. Animals were subjected to the chronic stress procedure for three consecutive weeks. Each week's stress regime included periods of food or water deprivation, intermittent illumination (lights on and off every 2 hours), cage tilting at a 45-degree angle, soiled cages (250 ml water in sawdust bedding), paired housing, low-intensity stroboscopic illumination (150 flashes/min), and periods with no stress. All stressors lasted for 10–14 hours continuously, day and night. The sucrose consumption test was conducted every Tuesday at 10.00 am for 24 hours during the three-week treatment period after stress induction. An increase in sucrose consumption was compared with the control group and expressed as mean ± S.D., with a p-value <0.05 considered significant.The results were presented as mean ± S.D. of the number of animals studied (n) observations and analysed using ANOVA, with significance set at $p < 0.05$.

Results:

Tail Suspension Test: Effect of fluoxetine, desipramine, ondansetron and buspirone (Table2). Both fluoxetine (10mg/kg; i.p.) and desipramine (15mg/kg; i.p.) significantly reduced the immobility time and increased the duration of struggle compared to the control group in TST, thereby suggesting an antidepressant like action($p<0.05$). On the other hand, both ondansetron and buspirone showed slight decrease in the immobility time, which was less than fluoxetine and desipramine and was not statistically significant.

Table 2. Effect of fluoxetine, desipramine, ondansetron and buspirone on immobility period of mice in TST, measured in seconds.

Groups	**Mean± SD(secs)**
1.Control (C)	182.00±8.48
2. Fluoxetine(F)	146.17±11.46*
4. Ondansetron(O)	166.50±6.05
5. Buspirone(B)	171.00±2.58
3. Ondansetron + Fluoxetine(OF)	126.17±7.30*
4. Buspirone + Fluoxetine(BF)	124.67±9.52*#

(* p value <0.05 when compared with control; # p value <0.05 when compared with fluoxetine)

Chronic Mild Stress Test: The chronic stress test model was developed by giving various stresses like tilting of cages, periods of food and water withdrawal etc. for the first 3 weeks. Chronic stress model caused a gradual decrease in the consumption of 1% sucrose solution every week, which was maximum at the end of 3rd week, thereby suggesting the development of depression. Thereafter drugs were administered in the next 3 weeks and the sucrose consumption was measured each week (Table 3 &4)

Table 3: Weekly change in stress response in chronic mild stress test model

	Control	Fluoxetine	Desipramine	Ondansetron	Grp F+O	Grp D+O
Week0	10.9±.3	15.2±.4	14.6±.6	12.5±.5	16.9±.4	16.5±.7
Week1	9.48±.4	12.60±.8	12.44±.8	10.75±.6	13.35±.6	13.89±.3
Week2	8.43±.6	10.65±.3	10.90±.5	9.50±.3	12.05±.4	11.23±.8
Week3	7.58±.5	9.60±.7	9.05±.3	8.65±.7	10.54±.3	10.50±.5
Week4	8.3±0.8	10.9±.7	11.4±.7	9.5±0.6	12.0±.4	11.3±.6
Week5	9.2±.7	13.4±.5	13.0±.8	11.0±.6	14.5±.5	13.9±.7
Week6	10.7±.6	15.9±.4*	15.0±.5*	12.5±.5*	16.3±.6*	16.3±.3*

Mean± S.D. in 24-hr sucrose consumption (in gm/kg) in Control(C),Fluoxetine(F), Desipramine(D), Buspirone(B), and Ondansetron(O) groups.(* p value < 0.05 as compared to the control)

Table 4. Effect of fluoxetine, desipramine, ondansetron and buspirone on sucrose consumption of mice in CST (gm/kg)

Groups	**Sucrose consumption(gm/kg) [Mean± S.D]**
Control (C)	10.7±.6
Fluoxetine(F)	15.9±.4*
Desipramine(D)	15.0±.5*
Ondansetron(O)	12.5±.5*
Ondansetron + Fluoxetine(OF)	16.3±.6*
Ondansetron + Desipramine(OD)	16.3±.3*#

(* p value <0.05 as compared to control)

Discussion:

Major depression is one of the most widespread psychiatric illnesses. It is an important public health problem since major depression induces disability, poor quality of life, economic burden or suicide. According to the monoaminergic theory of depression, deficiencies or imbalances in monoamine neurotransmitters, *i.e.*, serotonin (5-HT), noradrenaline (NA) and dopamine (DA), are involved in the pathophysiology of this disease.[18-20] New pharmacological strategies have emerged to improve efficacy and also, decrease the time for antidepressants to act. Since, SSRIs such as fluoxetine have

been shown to be of major benefit in the treatment of depression by enhancing the synaptic 5HT levels.[21] The mechanism by which the elevation of synaptic concentration of 5HT alleviates the symptoms of depression is not known but the involvement of multiple 5HT receptor subtypes would appear to be obvious factor.[22]

However, it is still not clear whether one is more important than the other. Also, several preclinical studies have suggested that targeting specific 5HT receptors with selective agonist and antagonist, may enhance the antidepressant response and reduce its delay compared to currently used antidepressants. It is still not clear that which 5HT subtypes are more important than others.

Tail Suspension Test

In the present study, when fluoxetine (10 mg/kg) and desipramine (15 mg/kg) were tested for their antidepressant effect, they significantly decreased the immobility time. While ondansetron (0.1mg/kg) when given alone, did not significantly decrease the immobility time. This finding is similar to the finding of Wallace A et al.[23]

In our study, ondansetron did not show antidepressant like action in the dose studied. This result was contrary to the effects seen by Guta D et al., [24]wherein it was found that ondansetron (0.5-2 mg/kg, i.p.) reduced the immobility time in mice in forced swim test.The possible explanation for such varied result is that the involvement of 5-HT3 receptors is complex and their molecular structure, function and regulation are only partially elucidated. Also, the various $5HT_3$ antagonist showed a bell-shaped dose response curve in preclinical studies. Moreover, in the present study, we have used only a single dose of ondansetron and buspirone due to constraint of animals. Therefore, further studies with higher doses need to be done to evaluate antidepressant action of ondansetron and buspirone in TST model.

In the interaction studies, it was found that all combination groups, i.e., ondansetron + fluoxetine, and ondansetron+ desipramine did decrease the immobility time significantly when compared to the control. While on comparing with the positive control, i.e., the fluoxetine group, the ondansetron + fluoxetine group did not cause significant decrease in the immobility time. Similarly significant antidepressant effect was not seen with ondansetron+ desipramine. This finding is similar to that found by G.P. Luscombe et al[25] wherein 8-OH-DPAT(3mg/kg, s.c.) was found to augment the effect of desipramine(3-30 mg/kg, s.c.) in the forced swim model of depression. The above findings suggest that ondansetron has a little role in augmenting the antidepressant effect of both fluoxetine and desipramine.

Chronic Mild Stress Test: Chronic sequential exposure of mice to a variety of mild stressors produces overall increase in depressive behaviours in rats and mice that appear similar to human depression. The most common behavioural change measured during CMS experiment is the presence of anhedonia represented by decreased consumption of 1% sucrose solution. The alteration caused by CMS is reversed by chronic treatment with traditional antidepressant drugs, desipramine and fluoxetine. In the present study, ondansetron significantly increased the sucrose consumption at the end of the 6-week experiment, as compared to control group. also, all combinations,i.e., fluoxetine+ ondansetron; and desipramine+ ondansetron significantly increased the sucrose consumption as compared to the control. On inter-group comparison, buspirone + fluoxetine and ondansetron + fluoxetine groups did not increase the sucrose consumption significantly more than the fluoxetine alone group.On the other hand, both the combinations of desipramine, i.e., desipramine+ buspirone and desipramine +ondansetron showed a significant increase in sucrose consumption than the desipramine alone group. Apart from these there are studies elucidating the interaction between SSRI and serotonin antagonists. One such study delves into the role of brain-derived neurotrophic factor (BDNF) in mediating the effects of SSRIs and their impact on developmental plasticity, shedding light on how SSRIs may interact with the serotonergic system.[26] Another study investigates the use of citalopram, an SSRI, in treating social phobia, this study explores how SSRIs may interact with serotonin receptors, providing insights into their potential mechanisms in anxiety disorders.[27] One paper critically analyses the relationship between serotonin and depression, highlighting a disconnect between popular advertising claims and scientific literature, emphasizing the complexity of the role

of serotonin in depression and the action of SSRIs.[28] Examining the potential of serotonin 1A (5-HT1A) agonists in depression treatment, the study byBiller P et al., explores their interaction with SSRIs and their potential contribution to enhancing the effectiveness of antidepressant therapies.[29]

Conclusion:

Our primary focus centred on the interaction between serotonergic modulators and two established antidepressants, fluoxetine and desipramine, within the context of albino mice as experimental models. In doing so, we aimed to uncover whether the addition of 5-HT3 antagonists, like ondansetron, might compromise or enhance the antidepressant effects of these medications.The prevailing wisdom often suggested that increasing serotonergic activity in conjunction with established antidepressants could lead to saturation and, paradoxically, a diminishment of therapeutic benefits. However, the results of our investigation challenge this notion, offering a tantalizing alternative perspective. In select animal models, it became apparent that the co-administration of 5-HT3 antagonists did not merely preserve the antidepressant effect of fluoxetine and desipramine but, intriguingly, appeared to enhance it.This unexpected finding underscores the intricacy of the serotonergic system and its role in mood regulation. While the serotonin hypothesis of depression has long guided our understanding of the condition, our study highlights that there may be hitherto unexplored pathways and interactions within this system that can be leveraged for more robust therapeutic outcomes.As we conclude our exploration, we must acknowledge the need for caution and further research. The effects observed in animal models may not directly translate to human populations, and the precise mechanisms underpinning this serotonergic synergy require in-depth investigation. Nevertheless, this discovery offers a promising avenue for future antidepressant drug development.

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