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Research Article

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# Biochemical, Neurochemical and Behavioural Responses Following Administration of 6-Fluoro-3-(Piperidin-4-Yl) Benzo[D] Isoxazole Derivatives and Antipsychotic Drugs in Mice

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**ABSTRACT**

The atypical antipsychotic drugs used in recent times for the treatment of chronic or acute psychotic (CNS) disorders, are found to cause Extrapyramidal side effects (EPS). Earlier studies have revealed that an enhanced oxidative stress accompanied with high glutamate transmission lead to extrapyramidal symptoms, thus limiting the use of established antipsychotic drugs. Therefore the current study investigates the effects of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives designated as S1, S2, S3, S4 as potent antipsychotics and to prove their efficacy in producing menial Extrapyramidal symptoms, by conducting biochemical, neurochemical and behavioural analysis. The behavioural studies on mice showed that chronic administration of standard antipsychotic drugs haloperidol (1 mg/kg *i.p*) and resperidone (1 mg/kg *i.p*) to animals have remarkably increased the vacuous chewing movements (VCM`s), VCM`s was noticeably inhibited in test compounds S2 and S3 (1 mg/kg *i.p*). Neurochemical analysis showed an increase in the concentrations of GABA, dopamine, and norepinephrine in the mice treated with synthesized molecules (S1-S4) compared to standard drugs. Alterations in the enzyme/protein levels of GSH, SOD, catalase and lipid peroxidation have further substantiated the role of free radicals as the underlying cause of EPS. Altogether it can be concluded that the study of synthetic molecules may yield a promising antipsychotic drug which can minimise EPS.

**Keywords:** Antipsychotic, Benzisoxazole, EPS, Free Radical, Dopamine.

## INTRODUCTION

Standard antipsychotic drugs used in the medication of psychotic disorders are restricted by its propensity to cause a range of extrapyramidal symptoms (EPS) like Parkinsonism, akathisia, dystonia and tardive dyskensia (TD). [1] EPS may be manifested after prolong treatment of antipsychotics and may persevere even after the withdrawal of the drug treatments, however the most concerning aspect of the disorders is the chances of reversibility to normal once EPS is manifested to be very feeble. [2] Manifestation of extrapyramidal symptoms is associated with increased concentration of central dopaminergic D2 receptors in experimental animals. [3] The metabolism of dopamine by enhanced glutamatergic transmission has experimentally revealed the involvement of free radicals in appearance of EPS. [4] The free radical generation accompanied by decreased antioxidant enzymatic levels also indicated extrapyramidal

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symptoms. [5] Moreover variations in catecholamine metabolism commonly observed after regular administration of antipsychotic drugs resulting in increased oxidative damage, which is experimentally correlated by decrease in concentration of neurochemicals such as dopamine, noradrenaline and GABA. [6]

In this line of investigation we have synthesized 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole derivatives, namely 4-(6-fluorobenzo[d]isoxazole-3-yl)-N-(3-methoxyphenyl)piperidine-1-carbothiamide(S1), N-(2-chlorophenyl)-4--(6-fluorobenzo[d] isoxazole-3-yl) piperidine-1-carbothiamide (S2), 4-(6-fluorobenzo[d]isoxazole-3-yl)-N-(2-fluorophenyl)) piperidine-1-carbothiamide (S3), N-(4-chlorophenyl)-4-(6-fluorobenzo[d] isoxazole-3-yl) piperidine-1-carbothiamide (S4). The designated molecules S1, S2, S3, and S4 were designed with piperidine moiety to interact with dopamine D2 receptor with lesser affinity and serotonin 5HT2a receptors with comparably higher affinity as possible drug molecules with fewer propensities to cause EPS. [7] In the present study we have investigated the effects of 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole derivatives (S1, S2, S3 and S4) and standard antipsychotic drugs on EPS along with related biochemical, neurochemical and behavioural responses.

## MATERIALS AND METHODS

### Animals

Swiss Albino mice (25 ± 5 g) of both genders were approved by Institutional Animal Ethics Committee (IAEC), under the rules 5(a) of the "Breeding and Experiments on Animal (control and supervision) rules 1998" [Ref: HSK Cp/IAEC, Clear/12013-14/121] after observing the usual formalities lay down by IAEC as per provisions made by CPCSEA. All the animals were placed in laboratory cages in an animal house maintained at 23 ± 2°C under standard light/dark cycle. All the animals had free access to standard food pellets and filtered water.

### Acute Toxicity Study

The dose selection was done out according to safe dose calculation as indicated in acute toxicity studies (OECD TG420) for the drugs and samples S1 to S4. Acute toxicity studies gave similar results like that of antipsychotic drugs. With this basis, we selected the dose 1 mg/kg for further studies.

### Drugs

Resperidone was procured from Risdon, Intas Lab, India, Haloperidol was procured from Senorm, Sun, India. DTNB, TBA and EDTA were from SD fine (India). All the other chemicals used were of analytical grade.

### Experimental design

Animals were divided into control and treated groups and each group had animals of both genders. They had free access to water and food. On the 22nd day, that is 24 h after the last *i.p* injection, the animals were utilized for biochemical, neurochemical and behavioural responses.

### Behavioural evaluation of vacuous chewing movements (VCMS)

Experiment was performed by dividing in 7 groups with six (n=6). All the test molecules including antipsychotics were administered (1 mg/kg *i.p*) to animals for animals for 21 consecutive days to induce vacuous chewing movements. [8] The behavioural evaluation was done after 24 hours of the last dose of the standard drugs and synthesized molecules (S1-S4). The each animal was placed in a small cage (30×20×30) specially designed for the evaluation of VCM`s. Back wall and the floor of the cage had mirrors to observe the VCM`s when animals faced on the opposite side of the observer. Animals were allowed to get acclimatised to the cage for 10 min before evaluation of the behaviour. VCM was observed as single mouth openings in the vertical plane and not related to physical aspect, even tongue protrusion or VCM observed during grooming was not recorded. The stereotypic behaviour was recorded continuously for 5 min. The observer was unaware about the treatment of drugs. [9]

### Biochemical analysis

Blood was collected from retroorbital plexus under menial anaesthesia on the 22nd day.

### Estimation of superoxide dismutase

Superoxide dismutase activity was determined based on the ability of SOD to inhibit the autooxidation of epinephrine to adrenochrome at alkaline pH. [10] Briefly, 0.1 ml of the supernatant obtained was added to a mixture of 0.1 mM epinephrine in carbonate buffer (pH 10.2) in a total volume of 1 ml and the formation of adrenochrome was measured at 295 nm. The SOD activity (U/mg of protein) was calculated by using the standard plot.

### Estimation of catalase

2.5 ml of phosphate buffer was added to 0.1 ml of serum and incubated at 25°C for 30 min. After transferring into a cuvette the absorbance was measured at 240 nm, 600µL of hydrogen peroxide solution was added to begin the reaction. The change in absorbance was measured for 5 min. [11]

### Estimation of reduced glutathione

0.5 ml of citrated blood and 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 min. To 0.1 ml of supernatant, 0.5 ml of DTNB and 1 ml of PBS were added. The absorbance was measured at 412nm. [12]

### Estimation of lipid peroxidation

0.1 ml of plasma was treated with 2 ml of 0.25N HCl, 0.35%TBA, and 15% TCA (1:1:1 ratio) and kept in the water bath for 20 min, the mixture was cooled and centrifuged and the clear supernatant obtained was measured at absorbance 535nm against the blank. [13]

### Simultaneous determination of noradrenaline and dopamine in brain by fluorimetric method

After behavioural evaluation (22nd day) all the animals were sacrificed and the brain was dissected out. Weighed quantity of tissue was homogenized in 0.1 ml hydrochloric acid - butanol, (0.90 ml of 37% HCl in one liter *n*-butanol) for 1 min in cold conditions. The homogenised sample was then centrifuged for 15 min at 2,000 rpm. 0.09 ml of supernatant was removed and added to an eppendorf tube containing 0.01 ml of heptane and 0.05 ml of 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the sample was again centrifuged under similar conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.03 ml) was used for estimation of Dopamine and noradrenaline. [14]

**Fluorimetric analysis of brain GABA**

The experiment was performed by sacrificing animals by decapitation and brains were quickly removed, weighed and placed in 5 ml of ice-cold trichloroacetic acid (10% w/v). The brain sample was then homogenized and centrifuged at 10,000 rpm for 15 min at 0°C. A sample (0.2 ml) of tissue extract was placed in 0.4 ml of 0.14 M ninhydrin solution in 0.5M bicarbonate carbonate buffer (pH 10), and then it was placed in a water bath at 55°C for 30 min, it was later cooled and treated with 5 ml of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). The measurement was done after 15 min at 455nm in a spectrofluorimeter was recorded. [15]

**Statistical Analysis**

All the data were expressed as mean ± Standard Error of Mean (S.E.M) and analysed using one-way analysis of variance (ANOVA) followed by turkey`s test. A probability of $P<0.05$ was considered to be significant.

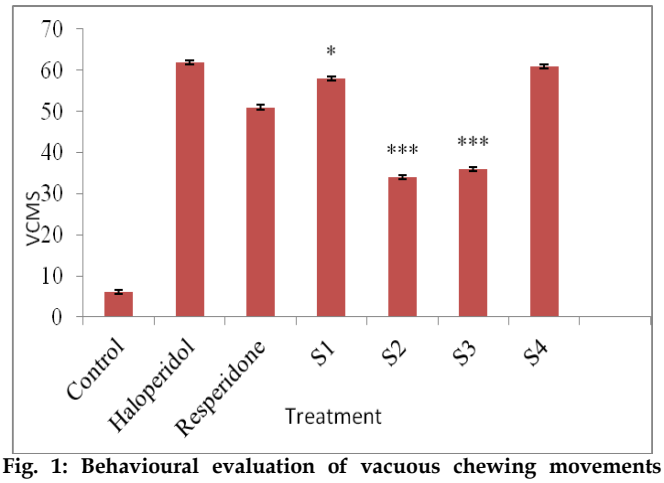


**Fig. 1: Behavioural evaluation of vacuous chewing movements (VCMS)**

a $p<0.001$,bp<0.01 statistically significant compared to control
* $p<0.001$ statistically significant compared to haloperidol
** $p<0.001$ statistically significant compared to resperidone
*** $p<0.001$ statistically significant compared to haloperidol and resperidone

## RESULTS AND DISCUSSION

**Behavioural evaluation of vacuous chewing movements (VCMS)**

Chronic administration of standard antipsychotic drugs (1 mg/kg) and synthesized molecules (1 mg/kg) for 21 consecutive days showed significant variations with respect to VCM`s recorded. VCM`s values for S2 and S3 was 32 and 36 respectively which was lower compared to haloperidol (62) and resperidone (51). In contrast the results obtained with S1 (58) and S4 (61) molecules were almost similar to standard drugs. The present results clearly demonstrated that the synthesized molecules S2 and S3.

**Biochemical analysis**

**Table 1: Studies of antipsychotic drug and test molecules on biochemical parameters**

| Treatment Dose [mg/kg] | LPO (nmoles/mg of protein) | SOD (U/mg of protein) | GSH (nmoles/mg of protein) | Catalase (micromole $H_2O_2$Degraded/mg protein/ min) |
|---|---|---|---|---|
| Normal (10 ml/kg, normal saline) | 12.13 ± 2.911 | 171.7 ± 0.51 | 7.028 ± 1.63 | 59.321 ± 0.52 |
| Haloperidol (1 mg/kg) | 16.09 ± 0.151 | 185.7 ± 0.17 | 3.42 ± 1.45 | 33.73 ± 0.37 |
| Resperidone (1 mg/kg) | 15.105 ± 0.356 | 182.5 ± 0.25 | 6.43 ± 1.24 | 48.25 ± 0.61 |
| S1 (1 mg/kg) | 15.96 ± 0.585* | 134.4 ± 0.37a** | 6.13 ± 1.26** | 46.74 ± 0.17* |
| S2 (1 mg/kg) | 13.13 ± 0.211** | 161.3 ± 0.41a** | 6.46 ± 1.16* | 68.26 ± 0.82a** |
| S3 (1 mg/kg) | 14.06 ± 0.235** | 171.6 ± 0.41a** | 8.512 ± 1.16a** | 63.67 ± 0.74a** |
| S4 (1 mg/kg) | 17.10 ± 0.156 | 166.1 ± 0.12a** | 5.27 ± 1.44* | 51.18 ± 0.27** |

a p<0.01 statistically significant compared to control
* p<0.01 statistically significant compared to haloperidol
** p<0.01 statistically significant compared to haloperidol and resperidone

**Estimation of Lipid peroxidation**

The results indicate that synthesized molecules S2 (13.13 ± 0.211) and S3 (14.06 ± 0.235) prominently decreased the amount of lipid peroxidation when compared standard antipsychotic drugs haloperidol (16.09 ± 0.151) and resperidone (15.105 ± 0.356), even S1 (15.96 ± 0.585) also showed relatively lower amount of peroxidation when compared to the standard drug haloperidol. Thus indicating decreased generation of free radicals in test molecules administered animals.

**Estimation of Superoxide dismutase**

SOD assay showed good results for all the synthesized molecules when compared to normal and the animals administered with standard drugs and normal (171.7 ± 0.51). S2 (161.3 ± 0.41), S3 (171.6 ± 0.41) and S4 (166.1 ± 0.12) showed almost similar results like that of standard drugs, whereas S1 (134.4 ± 0.37) showed lower values when compared to the above. The findings indicate decreased levels of free radicals in animals administered with synthesized molecules (S1-S4) this lead to fewer chances of causing EPS compared to antipsychotic drugs.

**Estimation of GSH**

The GSH values for standard drugs resperidone and haloperidol was 6.43 ± 1.24 and 3.42 ± 1.45, whereas higher values were seen in animals administered with S3 (8.512 ± 1.16), S2 (6.46 ± 1.16) and S1 (6.13 ± 1.26) when compared to animals administered with haloperidol. S4 (5.27 ± 1.44) also had higher values than haloperidol. Thus increased concentration of Glutathione indicates that lesser extent of oxidative stress is caused by the action of the drugs.

**Estimation of Catalase**

The concentration of protein was found to be high in S2 (68.26 ± 0.82) and S3 (63.67 ± 0.74) when compared to haloperidol (33.73 ± 0.37) and resperidone (48.25 ± 0.61) treated animals. The values of S2 and S3 treated groups were also found to be significant when compared to control. Thus higher catalase concentration supports lesser oxidative damage and menial chances of EPS.

Antipsychotic drugs are known to lower the gene expression of SOD, CAT and thus resulting in decreased enzyme levels and protein content. Since antioxidant enzymes play a vital role in maintaining physiological levels of free radicals, their role becomes more significant under oxidative stress. It is observed that higher generation of free radicals is accompanied with lower defence mechanism of antioxidants. The above study gave encouraging results for synthesized molecules when compared to standard antipsychotic drugs.

**Neurochemical evaluation**

**Table 2: Studies of antipsychotic drugs and synthesized molecules effects on brain catecholamines**

| Treatment Dose [mg/kg] | Dopamine (pg/mg) | Noradrenaline (pg/mg) | GABA (pg/mg) |
|---|---|---|---|
| Normal (10 ml/kg, normal saline) | 373.12 ± 0.26 | 348.23 ± 0.28 | 262.45 ± 0.58 |
| Haloperidol (1 mg/kg) | 175.82 ± 0.29 | 183.72 ± 0.42 | 138.56 ± 0.24 |
| Resperidone (1 mg/kg) | 198 ± 0.33 | 211.67 ± 0.74 | 182.38 ± 0.46 |
| S1 (1 mg/kg) | 237.34 ± 0.18** | 221.92 ± 0.34 | 178.43 ± 0.89 |
| S2 (1 mg/kg) | 463.23 ± 0.42a** | 442.87 ± 0.21a** | 312.32 ± 0.13a** |
| S3 (1 mg/kg) | 402.56 ± 0.74a** | 362.66 ± 0.12 | 288.35 ± 0.72** |
| S4 (1 mg/kg) | 207.43 ± 0.67 | 285.78 ± 0.25* | 191.54 ± 0.16 |

a p<0.01 statistically significant compared to control

* p<0.01 statistically significant compared to haloperidol

** p<0.01 statistically significant compared to haloperidol and resperidone

Chronic administration of test molecules and standard antipsychotic drugs has revealed significant results. Higher concentration of Dopamine, GABA and noradrenaline was seen in animals administered with S2 (463.23 ± 0.42, 442.87 ± 0.21, and 312.32 ± 0.13) and S3 (402.56 ± 0.74, 362.66 ± 0.12, 288.35 ± 0.72) compared to standard drugs haloperidol (175.82 ± 0.29, 183.72 ± 0.42, 138.56 ± 0.24) and resperidone (198 ± 0.33, 211.67 ± 0.74, 182.38 ± 0.46). Chronic administration of antipsychotics has been found to decrease the serotonin, norepinephrine and dopamine concentration in cortical regions of the brain. [17] This resulted in increase in the levels of dopamine receptors, thus decreasing the dopamine concentration, affecting the normal neurotransmission. [18] However in the present results increased concentration of dopamine caused by the synthesized molecules on treated animals indicates better antagonistic activity for dopamine D2 receptors compared to standard antipsychotic drugs.

In the present findings the newly synthesized molecules (S1-S4) has exhibited potent antipsychotic effects in mice. The results showed significant alterations in the levels of antioxidant enzyme/proteins such as SOD, GSH, catalase and lipid peroxidation. Dopamine, GABA and norepinephrine values of the synthesized molecules demonstrated their menial propensity to cause EPS. Behavioural response indicated by VCM`s also show promising potential of the synthesized molecules. Altogether it can be concluded that the synthesized molecules (S1-S4) have potential to develop into new drug molecules with tendency to cause lesser EPS.

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