Dizocilpine induced psychosis-like behavior in rats: A possible animal model with full spectrum of schizophrenia

Sidrah Shahzad[1], Saara Ahmad[2], Syeda Madiha[1], Saima Khaliq[3], Laraib Liaquat[1], Sadia Sadir[1], Sahar Rafiq[1], Saiqa Tabassum[1,4], Zehra Batool[1] and Saida Haider*[1]

[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan
[2]Department of Biological and Biomedical Sciences, The Agha Khan University, Karachi, Pakistan
[3]Department of Biochemistry, Federal Urdu University of Art, Science and Technology, Karachi, Pakistan
[4]Department of Biochemistry, Barrett Hodgson University, Karachi, Pakistan

Abstract: Schizophrenia (SZ) is categorized as neuropsychiatric disorder with reduced lifespan and significant impairments in social and vocational functioning. One of the best proposed pharmacological animal models is dizocilpine, as it can mimic the full spectrum of schizophrenic disorder including positive and negative symptoms along with cognitive deficits. Dizocilpine is N-methyl-D-aspartate (NMDA) receptor antagonist known to induce hyper-locomotion and stereotyped behavior in rodents. Present study was designed to develop an animal model of SZ via intraperitoneal administration of dizocilpine in rats (100-150g) at a dose of 0.3 mg/kg for eight days. For the evaluation of positive symptoms, hyperlocomotor behavior was monitored. Negative symptoms were assessed by sucrose preference test (SPT) and social interaction test (SIT). Moreover, Cognitive deficits were evaluated by novel object recognition test (NORT). After behavioral assessments animals were decapitated for further evaluation of biochemical and neurochemical estimations. Present findings revealed that dizocilpine injected rats exhibited significant hyperlocomotor behavior, depressive symptoms and cognitive deficits. Results are further strengthened with a marked increase in lipid per oxidation (LPO) in brain and a decline in reduced glutathione (GSH) levels. Biogenic amine levels (Dopamine, DA; 5-hydroxytryptamine, 5-HT) were also significantly increased and decreased respectively. Thus, present findings suggest that dizocilpine can be used as one of the best drug to develop psychosis-like symptoms in rats and to develop an animal model following a short-term study.

Keywords: Schizophrenia, dizocilpine, anhedonia, social withdrawal, oxidative stress.

INTRODUCTION

Schizophrenia (SZ) is defined as a chronic debilitating neuropsychiatric disorder associated with reduced lifespan followed by significant impairments in social and vocational functioning. It is a serious threat of brain disorder that affects approximately 1% of the total population worldwide (Tandon *et al*., 2010). Although the aetiology of this brain disorder remains contentious, however it is believed that it is one of the multifactorial neurodevelopmental disorders that has been influenced by both genetic as well as environmental factors. The full spectrum of SZ mainly cluster into three categories of symptoms: positive symptoms mainly comprised of visual and auditory hallucinations, delusions, thought disorder and conceptual disorganization, while negative symptoms consist of anhedonia, social withdrawal, emotional blunting, avolition and problems related to thought and content of speech. While the third category comprises of cognitive dysfunction followed by impaired abilities in working memory, executive function and attention (Roenker *et al*., 2012). It also has been reported that comorbid conditions of depression, anxiety and substance of abuse are also the hallmark of the disorder. Many neurotransmitter systems have been implicated in the pathophysiology of SZ including dopamine, serotonin, glutamate, GABA and acetylcholine. The earlier hypothesis invokes primarily dopamine abnormalities in the aetiology of the disorder. However, research in current years emphasizes the hypoactivity of the N-methyl- D-aspartate (NMDA), which are glutamate receptors and may be involved in the cause of development of SZ (Javitt *et al*., 2012).

Schizophrenic disease models play a significant role to analyse the underlying changes in the pathophysiology of the disorder and also to discover the new therapeutic strategies in order to treat the disorder. Dizocilpine, also designated as MK-801, is an uncompetitive antagonist of the NMDA receptor. It blocks the NMDA receptors via voltage dependent manner as the ion channel of the receptor must open for the drug to bind with it. Previous study has shown that MK-801 induces hyperactivity (Nilsson *et al.*, 2001), social flattening even at lower doses and most importantly a deficit in various cognitive functions (Stuchlík and Vales, 2005). Oxidative stress is another major hallmark of SZ. There is a huge growing evidence supporting that increased oxidative stress may contribute to the pathogenesis of SZ via generation of

*Corresponding author: e-mail: saida-h1@hotmail.com

increase reactive oxygen species in brain (Yao *et al*., 2011). In addition, decreased capacity of synthesizing glutathione (GSH) an antioxidant, also supports the appearance of oxidative stress (Gysin *et al*., 2007).

Although in previous studies dizocilpine has been used to develop SZ like symptoms. However, the present study was designed to analyse the full spectrum of SZ via single experimental and a short term study. In the present study, SZ like symptoms were not only monitored via different behavioral assessments but also biochemical and neurochemical estimations were performed to further confirm the development of the disorder.

MATERIALS AND METHOD

Experimental design

Twelve locally bred young male Albino-Wistar rats weighing 100-150 g (purchased from the HEJ research institute of Karachi) were used in the present study. Before starting the protocol, rats were allowed to acclimate for 1 week with free access to standard rodent diet and tap water. Rats (n=12) were randomly divided into two groups; first group designated as control (n=6) administered with saline (0.9% NaCl) and second group designated as test (n=6) administered with dizocilpine (0.3 mg/kg). Dizocilpine was purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). Drug was administered via intraperitoneal route for eight days. After this, behavioral assessments were performed and rats were decapitated for further analyses of biochemical and neurochemical estimations. All the treatment and behavioral assessments were performed in a balance design to avoid any error regarding order and time.

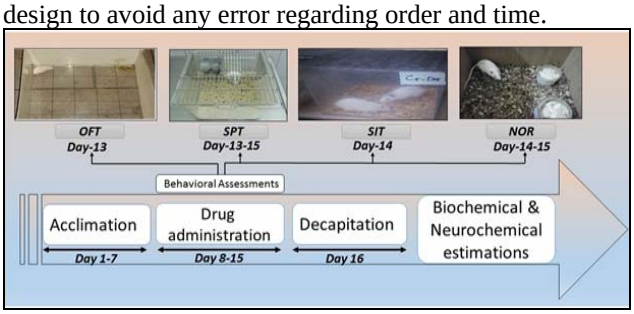

Fig. 1: Schematic representation of experimental protocol.

Behavioral assessments

Open-field test

To assess positive symptoms of SZ, hyper locomotion is one of the parameter for analyses (Barnes *et al*., 2015). The method for OFT was same as described previously (Haider *et al*., 2015).

Sucrose preference test (SPT)

SPT is used as an indicator of anhedonia which is described as lack of interest in presence of any reward or pleasurable state. It exists in few forms of affective disorder including depression. In SZ, anhedonia is one of the parameter to assess negative symptoms of the disorder (Tandon *et al*., 2009). This test was performed in order to assess rat's interest in seeking out a sweetened drink over plain drinking water. Since a bias towards the sweetened drink is normal and failure to do so is indicative of anhedonia or depressive symptoms.

Sucrose preference testing is carried out in home cage and cages were randomly allocated with two identical graduated water bottles for consecutive 3 days. One bottle contained plain drinking water while the second contained 250ml of 1-2% w/v sucrose solution (Kandratavicius *et al*., 2015). Sucrose solution and water intake was measured on daily basis and the position of the two bottles was switched after every 24hours to reduce any confound produced by a side biasness. After testing, sucrose consumption was calculated as volume of sucrose intake over total volume of fluid intake and converted into percentage.

Social interaction test (SIT)

A number of neuropsychiatric disorders are specified by disruptions in social behavior and social recognition such as in depression, bipolar disorders, autism spectrum disorders, obsessive-compulsive disorders and SZ (Le *et al*., 2005). To assess sociability in rats, SIT was performed. In SI test, behaviors are video-recorded and analyzed to assess active interactions (sniffing, following, boxing, crawling under or over, aggressive behavior, social grooming, chasing), passive interactions (close proximity with a distance of 5cm from skin to skin) and number of interactions of a test rat with a novel rat (Le *et al*., 2005). The test was performed after 24 h of injection of drug in a transparent activity box made of Perspex plastic having dimensions of (26×26×26cm) in a quiet room under bright illuminated white light. In each turn of testing, two rats were taken from two different groups for 5 minutes and rats were marked with different color paints for recognition. The total SI time was calculated which is the sum of time spent in active and passive interactions (Almeida *et al*., 2013). Less SI time exhibited by test rats is an indication of social withdrawal effects.

Novel object recognition test (NORT)

NORT was performed to assess the cognitive dysfunction in SZ since, this test is used to assess cognitive ability and recognition memory in rodents. This task is sensitive to disruption by NMDAR antagonist with good predictive validity (Neill *et al*., 2010) therefore, used in the present study to evaluate the cognitive symptoms of SZ. The testing procedure was same as described previously (Haider *et al*., 2016). In this test, time to explore (sniff, lick, chew) familiar and novel object was monitored for cut off time of 3 min. Preference index (PI) was calculated that is the ratio of time to explore novel object over total time to explore novel + familiar object.

Biochemical estimations

Determination of malondialdehyde (MDA) content and reduced glutathione (GSH)

Lipid peroxidation (LPO) and GSH levels were estimated by the same procedure as described by (Haider *et al*., 2016). LPO data was analyzed as µmol of MDA/g of brain tissue. GSH represented as µmol/g was estimated as explained earlier.

Neurochemical estimations

Determination of biogenic amines

Neurochemical estimation was performed to analyze the concentration of biogenic amines such as dopamine (DA) and 5-hydroxytryptamine (5-HT). The same method was followed as previously described by (Haider *et al*., 2016).

STATISTICAL ANALYSIS

Data values are presented as mean ± SD (n=6) and statistical differences were evaluated by independent sample *t*-test using SPSS version 16. Values of p less than 0.05 were considered as statistically significant.

RESULTS

Effect of administration of dizocilpine on behavioral assessments

Behavioral data presented in table 1 indicates the effects of dizocilpine administration in different behavioral assessments. In OFT dizocilpine treated rats exhibited hyperlocomotor behavior as evident from significantly ($p<0.01$) increased number of squares crossed as compared to control rats. Dizocilpine administered rats also showed social withdrawal effects as depicted via significant ($p<0.05$) decreased number of interactions, active and passive interactions ($p<0.01$) in SI test. Cognitive deficits were monitored by NORT, which showed that test rats exhibited significant ($p<0.01$) decreased PI as compared to controls.

Effect of dizocilpine administration on anhedonia

Anhedonia was evaluated by SPT. Behavioral results (fig. 2) revealed that dizocilpine treated rats exhibited decreased % sucrose consumption during 3 days of consecutive monitoring after 24 h (76.72±7.48%; $p<0.01$), 48h (76.49±11.81%: $p<0.05$) and 72h (63.59±6.91%; $p<0.01$) respectively.

Effect of dizocilpine administration on oxidative status of brain

In order to monitor oxidative status of dizocilpine treated rats as compared to control, LPO and GSH levels were analyzed. Dizocilpine injected rats showed a significant ($p<0.05$) increased concentration (158.12±29.68) of MDA in brain tissue. Dizocilpine treated rats also exhibited a significant ($p<0.05$) decline (91.91±15.48 µmol/g) in GSH levels as compared with saline treated rats (141.05± 38.46 µmol/g) as demonstrated in fig. 3.

Table 1: Effect of dizocilpine (MK-801) on behavioral assessments: OFT, SIT and NORT.

Behavioral Assessments	Parameters	Control	Dizocilpine
Open-field test (OFT)	Number of Square crossed	111.67±13.8	427.5±86.25**
Social interaction test (SIT)	Number of interactions	16±3.03	12.16±2.54*
	Active interaction	26.9±5.62 sec	18.75±5.26 sec**
	Passive interaction	15.33±2.21 sec	9.22±1.43 sec**
	Total Social interaction(SI)	42.27±6.85 sec	28.5±4.87 sec**
Novel object recognition test (NORT)	Sniffing time (Novel)	7.16±1.16 sec	4.77±0.77 sec**
	Sniffing time (Familiar)	4.05±0.64 sec	5.05±0.48 sec*
	Preference index (PI)	0.63±0.063	0.48±0.03 **

Values are represented as mean ± SD (n = 6). Mean differences were evaluated by independent sample t-test using SPSS version 16. Statistical difference is represented by *p<0.05 or **p<0.01.

Effect of dizocilpine administration on biogenic amines

The analysis of biogenic amines showed that the test rats exhibited a significant ($p<0.01$) increased levels of DA (176.35±8.41ng/g) in brain tissue compared to control rats (60.40±12.40ng/g) as depicted in fig. 4. There was a significant decline ($p<0.01$) in levels of 5-HT (4.56±0.97 ng/g) following with dizocilpine administration as compared with control group (16.76±1.01ng/g) as shown in fig. 4.

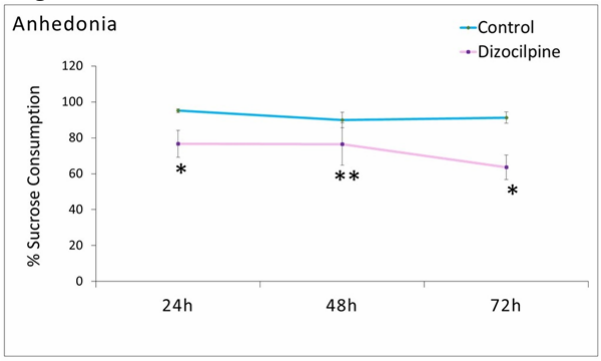

Fig 2: Effect of dizocilpine administration on state of anhedonia was evaluated by % sucrose consumption at 24 h, 48h and 72h performed in sucrose preference test (SPT).

Values are represented in the form of %mean ± SD (n=6) and differences between control and test group were evaluated by independent sample t-test. *p<0.05 or **p<0.01 was taken as statistically significant.

DISCUSSION

The present results showed that dizocilpine is one of the best drugs that can mimic the full spectrum of SZ. In order to better understand the pathophysiology and the underlying mechanism associated with the symptoms of this disorder, present study was conducted to develop a possible animal model of SZ via short term study. Present study is also concomitant with the previous studies (Nilsson *et al*., 2001) in the development of SZ like symptoms in rodents at different parameters. Present

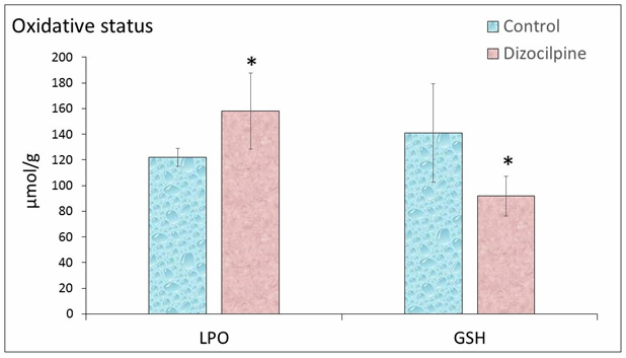

Fig 3: Oxidative status in whole brain of dizocilpine treated rats and control rats was monitored by evaluation of lipid peroxidation (LPO) in terms of malondialdehyde (MDA) levels and reduced glutathione (GSH) represented in µmol/g of brain tissue. Values are mean ± SD (n = 6) and statistical difference is represented as *p<0.05.

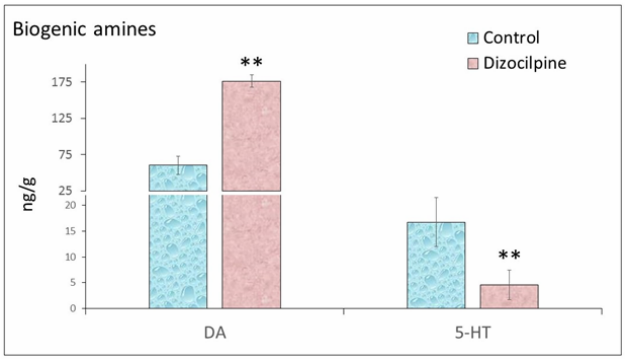

Fig 4: Biogenic amine levels in whole brain were estimated by levels of dopamine (DA; ng/g of brain tissue) and 5-hydroxytryptamine (5-HT; ng/g of brain tissue) as depicted in fig-3.
Values are represented as mean ± SD (n = 6) and statistical difference is represented as **p<0.01.

findings demonstrated that dizocilpine induced a full spectrum of SZ. Positive symptoms revealed from OFT that exhibits hyperactivity (Xiu Y *et al*., 2014). This finding is further confirmed by neurochemical estimation of DA which demonstrated increased concentration of DA in brain. Since, the earlier hypothesis predicted that increased DA tone in brain leads to positive psychotic symptoms of SZ (Schwartz *et al.,* 2012).

Next to positive symptoms, negative symptoms are also another hallmark of the disorder (Roenker *et al.,* 2012). Negative symptoms in the present study were examined by SPT which exhibits state of anhedonia following dizocilpine administration, as observed by less preference towards sweetened water over regular water. Social withdrawal effects are also associated with the negative symptoms of SZ (Roenker *et al.,* 2012). Present study assessed these effects via SIT, which demonstrated social withdrawal symptoms as recognized by reduced total SI time shown by test rats in comparison with control rats.
Moreover, dizocilpine treated rats also disclosed cognitive deficits, as evident from less time spent in sniffing of novel object as compared to a familiar one, this represents a decline in recognition memory in NORT, since, the third category of SZ symptoms are related to cognitive deficits (Porsolt *et al.,* 2010). Present findings were also in accordance with the previous studies in showing memory impairment effects following dizocilpine treatment (Liu *et al.,* 2017). These findings in our study are further justified by neurochemical estimation of 5-HT, that depicted decreased concentration of 5-HT in whole brain. As a decrease in levels of 5-HT is correlated with the negative and cognitive decline in SZ (Švob *et al.,* 2016) therefore, decline in 5-HT levels elicit depressogenic (Cowen *et al.,* 2015) and memory impairing effects (Buhot *et al.,* 2000).

Furthermore, present data also suggested that increased oxidative stress occurs in response to dizocilpine administration as shown by increased levels of MDA which is a marker of oxidative stress. These findings were further strengthened by decreased levels of antioxidant (GSH) observed in test rats in response to higher oxidative damage. The present findings further support the former studies (Yao *et al.,* 2011 and Gysin *et al.,* 2007) that increased oxidative damage may contribute towards the pathogenesis of SZ via generation of free radicals in brain and decreased potential of synthesizing antioxidants.

CONCLUSION

Therefore, it has been concluded from the present acute findings that dizocilpine induced SZ-like symptoms of all major categories in rats as evident from behavioral, biochemical as well as via neurochemical estimations. Moreover, this study also correlates the schizophrenic symptoms with development of an oxidative stress. However, further brain histopathological studies are needed for analyses of anatomical changes in dizocilpine-induced animal model of SZ.

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