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Evaluation of Drug Induced Neuronal Damage in Rats

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ABSTRACT

Previous somatic treatments of schizophrenia include continuous sleep therapy, insulin coma, electroconvulsive therapy & psychosurgical treatments. These options are usually associated with serious adverse effects. Over the ensuing 50 years, several classes of compounds with anti-psychotic activity have been developed. Fluphenazine is one of the typical antipsychotic drugs, use to treat schizophrenia and other mental disorders.

The present study was designed to evaluate various neuronal damages & behavioral impairment caused by the intramuscular administration of fluphenazine deconate in the rats. Rats were treated with 5mg/0.2ml fluphenazine IM for one month. This research work revealed a definite pattern, sequence and manifestation of the adverse effects produced by the drug during the whole period. Fluphenazine treated rats have shown a significant decreased loco motor activity, some neuronal damage & cognitive impairment, as rats developed rotational behavior & ptosis after the chronic administration of the drug which is a clear cut indication of neuronal stress as compare to the control rats. The inhibition of the locomotor activity is may be due to decreased level of dopamine, GABA ergic hypofunction & oxidative stress at basal ganglia. Cognitive impairment & rotational behavior in fluphenazine treated rats clearly revealed that this drug may impart neuronal damage in rats.

Keywords: Schizophrenia, typical antipsychotic drugs, Fluphenazine Deconate, Extra pyramidal effects, Dopamine

1. INTRODUCTION

Antipsychotic agents in the central nervous system have been considered to play an important role in modulation of mental diseases. Antipsychotics work by blocking dopamine receptors along with three pathways, and over the long-term, these pathways become increasingly dysfunctional (at least in a high percentage of patients.) The dysfunction in the basal ganglia leads to tardive dyskinesia. The dysfunction in the limbic system and the frontal lobes leads to tardive psychosis and tardive dementia.[1] Beside their beneficial effects these drugs shown severe potential side effect like neuronal damage, cognitive impairment, impaired loco motor activity, tardive dyskinesia, dystonia and other extra pyramidal effects. Various clinical[2,3] and experimental[4] studies have been carried out to study the effects of different neuroleptic drugs which are considered to affect via neurotransmitter systems[5].The clinical results about the mechanism of these drugs are continuing up to now[6,7]. More recently, investigators have postulated that neuroleptic medications may cause effect on motor system through mechanism other than direct dopaminergic receptor blockade. There is a possibility that neuroleptic medications may exert an effect on motor system through the creation of free radicals[8.] The hippocampal region is an essential component of learning and memory processes[9] and has been shown to be damaged by a number of toxicants[10,11,12] some of which are neuroleptic agents[13] Previous studies shows that fluphenazine has a tendency towards deterioration in the phase of memory consolidation[14].

Fluphenazine is a phenothiazine derivative used to treat schizophrenia and mental disorder; it blocks postsynaptic dopamine receptors in the mesolimbic system and increase dopamine turnover by blockade of DA somatodandritic autoreceptor[15]. Fluphenazine differs from some phenothiazine derivatives in several aspects: it has less potentiating effect on CNS depressants and anesthetics than some of the phenothiazines and appears to be less sedating[16]. But long-term administration of fluphenazine may contribute to the drug-induced antipsychotic extra pyramidal effects[17].

In the present study we attempted to evaluate the behavioral changes and possible neuronal damage in rats by fluphenazine deconate. For this purpose drug was administered for a period of one month and observed different behavior of rats by means of experimental parameters.

2. EXPERIMENTAL

The present study was carried out on 20 locally bred Swiss albino rats weighing from 150– 200 gram. All animals were equally divided into 2 groups each comprising of 10 animals, one group was served as control and the second group was Fluphenazine deconate treated. The animals were housed in standard sized polypropylene cage in pair and are maintained in reserve light and dark cycle of 12 hour.

Fluphenazine deconate is available as 25mg/ml in ampule form, each rat was given a fixed dose of 0.2ml/day containing 5ml of drug intramuscularly, while control animal were given saline daily. All the animals were maintained under constant environment condition 21 ± 1°C and humidity (50-60%).

2.1 *Experimental parameters*

All parameters were carried out in balanced design to avoid the order effect. Various experimental parameters were

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monitored during 30 days treatment and are given below

2.1.1 *Cage Crossing Activity*

Apparatus: Specifically designed Perspex cages (26×26×26cm) with Sawdust covered floor were used to monitor the cage crossing activity.

Procedure: The experiment was conducted in separate quite room. Both control and treated rats were placed in separate activity boxes. Rats become familiarized with their environment during this period. Then rats were observed for number of cage crossing within 5 minutes.

2.1.2 *Open Field Activity*

Apparatus: The open field apparatus used in the present study consisted of a square area (76×76cm) with walls 42cm high. The floor of apparatus was divided by lines into 25 squares.

Procedure: Experiment was conducted in a quiet room and white light. To determine open field activity, an animal is taken out from the home cage and was placed for first time in the center of open field apparatus. Number of squares crossed with all four paws and latency to move from the center square was scored for 5 minutes.

2.1.3 *Traction*

Apparatus: The apparatus consist of especially designed metallic wire / rod in order to monitor the gripping activity of rat.

Procedure: The rat was forced to hang on a wire / rod and the time for which rat hang the wire / rod was noted.

2.1.4 *Exploration*

Apparatus: Especially designed box having small holes like windows at a specific distance were used to monitor exploratory activity of rats.

Procedure: Animal was taken out from its home cage and placed in especially designed box. The animal tried to escape from these opening and dipped its head through these openings, the number of head dipped was counted for each rat for the period of 5 minutes.

2.1.5 *Gross behavior*

All the animal were observed for acute behavioral changes of the drug, these include grooming, Straub's phenomenon, Writhing reflex, tremors, twitches, righthing, reflex, pinna reflect, corneal reflex, anapthalamus, salivation, lacrimation, defecation and urination etc.

3. STATISTICAL ANALYSIS

Results are presented as mean ± standard. Comparison of difference of mean between control and Fluphenazine deconate treated group was made by using student's t-test. *P* value less than 0.05 were considered statistically significant and *p* value less than 0.005 were considered highly significant.

4. RESULTS

4.1 *Effect of fluphenazine deconate on Gross behavior*

Table1. Shows variety of acute gross behavior changes after administration of fluphenazine deconate during 30 days. The result observed were tremors, hind limb abduction, ptosis, muscle weakness, grooming, rotational behavior, flaccid tail and neck and statue position etc.

Table-1: Observation on Gross Behavior

Days of administration	**Control**	**Treated Fluphenazine**					
		5	**10**	**15**	**20**	**25**	**30**
Parameters							
Twitches	Normal	No Change	No Change	-	-	-	-
Ptosis	-	-	-	-	-	-	-
Hind paw abduction	-	+++	+++	+++	+++	+++	++++
Flacial Passivity	-	++	++	++	+++	+++	++++
Pinna reflex	-	-	+	+	+	+++	++++
Corneal reflex	-	-	+	+	+	+	++
Exopthalamus	-	-	-	-	-	-	-

Anopthalamus	-	-	-	-	-	-	-
Salivation	-	No Change	No Change	No Change	No Change	No Change	No Change
Lacrimation	Normal	No Change	No Change	No Change	No Change	No Change	No Change
Defecation	Normal	No Change	No Change	No Change	No Change	No Change	No Change
Urination	Normal	No Change	No Change	No Change	No Change	No Change	No Change
Motor activity	Normal	+	++	++	++	++	+++
Head Rotation	Normal	+++	+++	+++	+++	+++	++++

4.2 *Effect of Fluphenazine deconate on Cage Crossing and Open Field Activity*

Fig-1. Shows the effects of Fluphenazine deconate on cage crossing & open field activity on rats. Statistical analysis by two-tailed t-test revealed that fluphenazine deconate decreases both activities ($P<0.01$) which were highly significant.

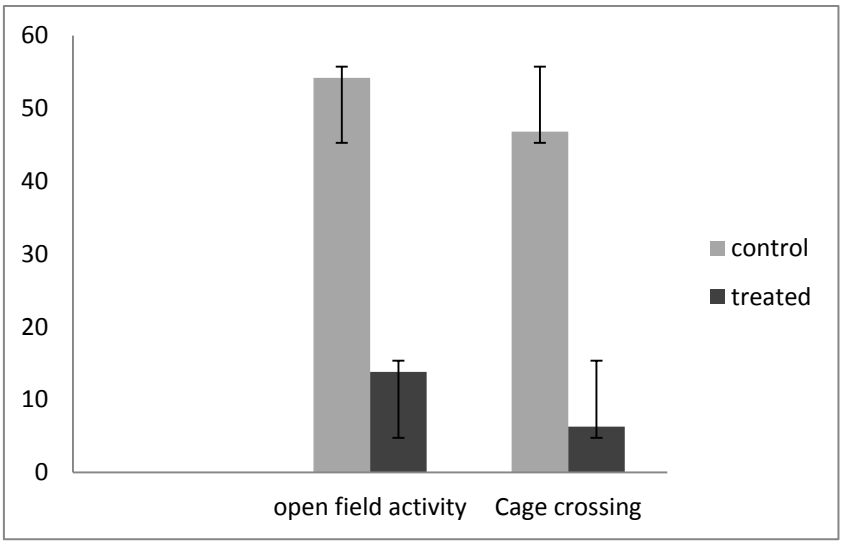

Fig-1: Effect of Fluphenazine on Open field & Cage crossing activity

4.3 *Effect of Fluphenazine deconate on Traction activity & Exploratory behavior*

Fig-2. Shows the effects of Fluphenazine deconate on traction activity and exploratory behavior on rats. Statistical analysis by two-tailed t-test revealed that fluphenazine deconate decreases both activities ($P<0.05$) which were significant.

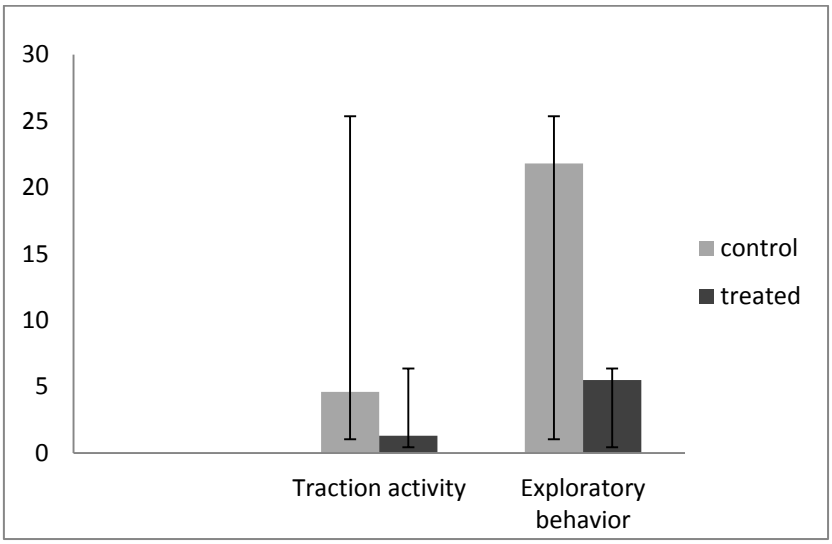

Fig-2: Effect of Fluphenazine on Traction activity & Exploratory behavior

4.4 *Effect of Fluphenazine deconate on Forced swimming activity*

Table-2. Shows the effects of Fluphenazine deconate on forced swimming activity of rats. Statistical analysis by two-tailed t-test revealed that fluphenazine deconate decreases forced swimming activity $P<0.05$) which were highly significant.

Table-2: Effect of Fluphenazine on Swimming Induced Activity in Rats

Drugs	Swimming induced activity
Control	143.1 ±3.17
Fluphenazine deconate	13.8**±2.74

Values are mean ± S.E (n=10)
P<0.05* =Significant
P<0.01** =Highly Significant

5. DISCUSSION

There are lot of experimental studies about useful effect of neuroleptic agents such as neuroprotective and neurogenesis at microscopic level[18]but it has been mentioned by only some researcher that treatment with these agents have some side effects such neurodegeneration or neuronal cell loss[19]in some area of brain, for examples hippocampus, striatum, medial prefrontal cortex.

Our study was limited to only one time point for treatment (30 days). The study was designed to evaluate neurochemical and behavioral effect of fluphenazine in rats. In present study, Fig-2 Shows significant decreased motor activity in an open field. Fluphenazine treatment induced hypo locomotion is mediated by nigral dopaminergic dysfunction[20] producing few effects on peripheral movements, grooming, immobility and defecation on open field. It was proposed that changes in the activity of the noradrenergic system in the amygaloid cortex may be casually related to the changes in the activity of the rats in the open field apparatus and can be related to the effect on adenosine which contributes to this effect [20].

In the present study, Fluphenazine showed a significant effect in home cage activity as shown in Fig-2. This may be due to dopamine inhibitory action. Since hyperactivity is often associated with decreased DA and decreased AC at D2 levels. This could be related to the fact that dopamine reduces the influence of the indirect pathway and increase the action of the direct pathway within the basal ganglia[21].

Our results indicate that Fluphenazine reduced DA concentration and effect on behavior as we have reported in Table-1. It was reported by Hatib et al.[22] that there is decreased 5HT, NE and DA in the stratum, olfactory tubercle and cerebral cortex, with corresponding elevated metabolite levels within 24 hours which could be seen after the administration of anti-psychotics which is consistent with our findings also.

Fluphenazine induced orofacial dyskinesia, a syndrome related to nigrostriatal dopamine supersensitivity[23]. Oxidative stress has also been increased during dopamine metabolism in brain. A number of free radicals are formed especially iron free radicals are formed which are quite toxic for neurons and neuronal damage may lead to a state similar to the Parkinson disease called pseudo Parkinsonism[24] which is clearly observed during the present study. Drug treated animals exhibited complete loss of exploratory behavior and complete passivity in traction and exploratory activity as Fig # 1 shows these effect, this is due to dopamine which produce significant impairment of cognitive function and loss of sedation due to severe sedation caused by fluphenazine. Also this effect could be due to decreased conductance of Na^+ ion as reported by Xiaoping et al[25].

There was no increased in struggling time during forced swimming test as we can see in Table-2 that clearly indicates the fluphenazine does not increase the desire for survival and has no anti- depressant profile. The antidepressant action is due to amine neurotransmitters and according to Frank et al,[24] the transporter proteins require to inactivate synaptically released dopamine and serotonin resist adaptation, to long term treatment with noval anti-psychotics that effect neurotransmission by these amines.

There was more salivation in experimental animals and the head was uncontrollably moving and very significantly hind paw abduction was noted that indicate the extent of neuronal damage. Few rats developed rotational behavior which is clear cut indication of neuronal stress. D4 receptors can be unregulated by nigrostriatal dopaminergic denervation, which suggest that unlike D1 and D2 receptors do not play a pivotal role in rotational behavior in rats with unilateral dopaminergic lesions[26]. Ptosis was very significant due to neuronal damage.

Limbic system is also affected probably which is called dopamine rich area and according to[27] there is a common role for medial prefrontal cortical D2 and striatolimbic D4 receptors in mediating clinical response of typical and atypical anti-psychotics.

One or more animals developed very characteristic features such as they develop an externally imposed posture. Catalepsy was significant in fluphenazine treated animals as compared to control groups.

6. CONCLUSION

Present study was planned to see any neuronal damage produced by the drugs, CPZ like fluphenazine deconate produces characteristics symptoms in rats i.e ptosis, muscular weakness, head drop, abused hind limb. Our results are in accordance with above findings; different experimental parameters were used to check the activity of rats like cage crossing, open field, forced swimming, traction and exploration. Drug treated rats shown a significant decreased

locomotor activity in these experimental parameters as compare to the control rats. The main reason of decreased locomotor activity is due to the decrease level of dopamine, GABA ergic hypofunction, excite toxicity and oxidative stress. It was also found that weight and food intake was significantly decreased after the drug administration. There was cognitive impairment and rotational behaviors in rats after the administration of fluphenazine deconate which clearly revealed that fluphenazine may produce neuronal damage in rats. In clinical settings, should be avoided from high dose fluphenazine treatment.

7. REFERENCES

1. Crane, G., Tardive Dyskinesia in Patients Treated with Major Neuroleptics. American Journal of Psychiatry**. (1968)** 124, supplement: 40-47.
2. Hirsch, S. R., Kissling, W., et al. A 28-week comparison of ziprasidone and haloperidol in outpatients with stable schizophrenia. J Clin Psychiatry. **(2002)** 63: 516-23, http://dx.doi.org/10.4088/JCP.v63n0609.
3. Labelle, A., Light, M., et al. Risperidone treatment of outpatients with schizophrenia: no evidence of sex differences in treatment response. Can J Psychiatry. **(2001)** 46(6):534-41.
4. Broerse, A., Timmerman W. et al. Antipsychotics and single-cell activity in the rat superior colliculus. Neuropsychopharmacol Biol Psychiatry. **(2002)** 327-33
5. Harrison, P. J., The neuropathological effects of antipsychotic drugs. Schizophr Res. **(1999)** 40(2): 87-99, http://dx.doi.org/10.1016/S0920-9964(99)00065-1.
6. Naidu, P. S., Singh A, Kaur P. et al. Possible mechanism of action in melatonin attenuation of haloperidol-induce dorofacial dyskinesia. Pharmacol Biochem Behav. **(2003)** 74(3): 641-8, http://dx.doi.org/10.1016/S0091-3057(02)01051-1.
7. Wakade, C. G., Mahadik, S. P., et al. Atypical neuroleptic stimulate neurogenesis in adult rat brain. J Neurosci Res. **(2002)** 69(1): 72-9, http://dx.doi.org/10.1002/jnr.10281.
8. Jamws, B. L, Michael, P., et al. Neuroleptic-induced striatal damage in rats. Psychopharmacology **(2000)** 148:171:179.
9. Korbo, L., Ladefoged, O., et al. Neuronal loss in hippocampus in rats exposed to toluene. Neurotoxicology**. (1996)** 17(2): 359-66.
10. Lawston, J., Borella, A., et al. The excitotoxicity of heterocyclic dicarboxylic acids in rat hippocampal slices: structure-activity relationships. Brain Res. **(1992)** 571(1): 145-8, http://dx.doi.org/10.1016/0006-8993(92)90521-A.
11. Pena, F., Tapia, R., Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: role of glutamate- and GABA-mediated neurotransmission and of ion channels. Neuroscience. **(2000)** 101(3): 547-61, http://dx.doi.org/10.1016/S0306-4522(00)00400-0.
12. Walsh, T. J., Emerich, D. F., The hippocampus as a common target of neurotoxic agents. Toxicology. **(1988)** 49(1): 137-40, http://dx.doi.org/10.1016/0300-483X(88)90185-0.
13. Gepdiremen, A., Aydin, N., et al. Chronic treatment of haloperidol causes vasoconstriction on basilar arteries of rats, dose dependently. Pharmacol Res. **(2004)** 50(6): 569-74, http://dx.doi.org/10.1016/j.phrs.2004.06.003.
14. Roussinov, K. S., Yonkov, D., Comparative studies on the effect of lithium and haloperidol on learning and memory, Acta Physiol Pharmacol Bulg**. (1975)** 3-4: 51-7.
15. Crane, G., Clinical Pharmacology in its 20th year. Science **(1973)** 181. 124-128, http://dx.doi.org/10.1126/science.181.4095.124.
16. Harrison, P. J., Weinberger, D. R., et al. Schizophrenia genes and neuropathology. Molecular psychiatry, **(2005)** 10, 40-68, http://dx.doi.org/10.1038/sj.mp.4001558.
17. Gale, K., Chronic blockade of dopamine receptors by antischizophrenic drugs enhances GABA binding in substantianigra. Nature. **(1980)** 283: 569- 570, http://dx.doi.org/10.1038/283569a0.
18. Wakade, C. G., Mahadik, S. P., et al. Atypical neuroleptic stimulates neurogenesis in adult rat brain. J Neurosci Res, **(2002)** 69(1): 72-9, http://dx.doi.org/10.1002/jnr.10281.
19. Bardgett, M. E., Humphrey, W. M., et al. The effects of excitotoxic hippocampal lesions in rats on risperidone- and olanzapine-induced locomotor suppression. Neuropsychopharmacology. **(2002)** 27: 930-8, http://dx.doi.org/10.1016/S0893-133X(02)00376-7.
20. Shiozaki, S., Ichikawa, S., et al. Action of adenosine A2A Receptor antagonist KW-6002 on drug induced catalepsy and hypokinesia caused by reserpine. Psychopharmacology **(2000)**.
21. Clow, A., Jenner, P., Theodorou, A., et al. Striatal dopamine receptors become supersensitive while rats are given trifluoperazine for six months. Nature; **(1979)** 278: 59-61, http://dx.doi.org/10.1038/278059a0.
22. Hatip-Al-Khatib, I., Mishima, K., et al. Microdialysates of Amines and metabolites from core nucleus accumbens of freely moving rats **(2001)**.
23. De Leon, J., The Effect of Atypical versus Typical Antipsychotics on TardiveDyskinesia. Eur. Arch. Psychiatry Clinical Neurosciences. **(2007)** 257: 169-172, http://dx.doi.org/10.1007/s00406-006-0705-z.

24. Frank, I., Tarazi,. et al. Olanzapine, quetiapine and resperidone: Long term effects on monoamine in rats forebrain. Neuroscience letters. **(2000)** Vol.278 (issue 2) pgs 81-84
25. Zhan, X., Dong, X. W., The neuroleptic drug, Fluphenazine blocks neuronal voltage-gated Na channels. Brain-Research**, (2006)** Vol.: 1106, issue 1, Pg # 72-81.
26. Zhang, K., Trank, I., et al. Nigrostriatal dopaminergic derivation enhances dopamine D4 receptor binding in rat caudate-putamen. Pharmacology Biochemistry and behavior, **(2001)** Vol-69, Issue 1-2, May-June. Page# 111-116
27. Frank, I., Tarazi., et al. Effect of nigrostriatal dopamine deviation on ionotropic glutamate receptors in rats caudate-putamen brain research. **(2000)** Vol: 881.Pg 69-72.