



Effect of Haloperidol on Development of Hepatocytes in the Albino Rats

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ABSTRACT

Introduction: Haloperidol is first generation antipsychotic used to treat psychosis. Its usage during pregnancy benefits psychotic mother and is indispensable for treating psychiatric emergency situations. **Aims & Objectives:** To evaluate the effects of haloperidol on development of liver given to albino rats in intrauterine period. **Place and duration of study:** Animal house PGMI, Bird wood Road. Duration of study is 2 months. **Material & Methods:** 45 female albino rats and 12 male rats of Sprague-dawley strain were used for conception. After conception, female rats were randomly divided into three groups A, B and C. Each group having 15 rats. Group A did not receive any drug whereas group B & C were given intraperitoneally haloperidol in a dose of 0.4mg/kg and 0.8mg/kg respectively. Hysterotomy was done on 21st day of gestation and pups were removed. Average litter size of pups in each group was 5-10 pups per female albino rat. These pups were labeled as A1, B1 and C1. They were grossly examined for any abnormality and liver was removed after dissection. Slides were made and stained to evaluate changes in detailed histological study of hepatocytes. **Results:** Comparison of diameter of hepatocytes in group B1 and C1 were significant from group A1 with p-value ≤ 0.001 . However comparison of hepatic nucleus diameter among three groups were statistically insignificant with p-value 0.357. **Conclusion:** Haloperidol was responsible for destroying the normal architecture of the developing liver. Liver growth was shown by poor development of hepatocytes at high doses.

Key words: Haloperidol. Developing fetus. Liver development. Teratogenic in pregnancy.

INTRODUCTION

Psychosis is a disease of neuronal disconnection caused by multiple genetic and environmental factors that affect brain development¹. The genetic etiology is likely to be polygenic. Approximately 6.6 percent of all first degree relatives of an affected individual can be affected by psychosis². Psychosis treatment involves education, medication, close monitoring of symptoms, stress management and creating a strong, supportive environment. Medication is important in relieving symptoms of psychosis and is critical in preventing relapses. They are called antipsychotics or neuroleptics³. Haloperidol was approved by the United States Food and Drug Administration (FDA) on April 12, 1967⁴; it was later marketed in the U.S. and other countries under the brand name Haldol by McNeil

Laboratories. Haloperidol is a first-generation (typical) neuroleptic, non-selective and binds to a broad range of receptors as dopamine D1 and D2, 5-HT₂, histamine H₁ and α ₂ adrenergic receptors in the brain. Its mechanism of action is due to antagonism of dopamine receptors in the mesolimbic and mesofrontal systems⁵.

The monitoring of haloperidol is important clinically, however large doses can be given safely in intravenous and intramuscular injections for rapid neuroleptization⁶. Plasma haloperidol concentration varies and its hydroxyl metabolites are responsible for biological activity and prolonged clinical effects. The recommended dose in psychosis is 0.5 to 5 mg twice or thrice, intramuscular doses vary from 2 to 30 mg whereas intravenous up to 30mg⁷. Lethal dose (LD50) in rats is ≥ 128 mg/kg subcutaneously, 27mg/kg intraperitoneally and 15mg/kg intravenously⁸. Haloperidol is contraindicated in

coma, acute stroke cardiac disease, pregnancy and lactating mother.

Various studies were conducted in the past to evaluate effects of haloperidol in different organs of adults and fetus. In a series of paper by Lewis et al, he concluded that haloperidol stunted brain growth⁹. In 1990, it was suggested that forebrain development was affected¹⁰ with its usage and proliferation of brain cells were affected during development¹¹. In 2002, increase no of apoptotic cells were seen in cerebral cortex¹² and vasoconstriction of basilar artery was observed in 2004 with chronic haloperidol usage¹³.

Damaging effects on adult liver was observed in a study conducted in 2009¹⁴. In 2010, another study conducted on guinea pigs also revealed damaging effects of haloperidol on adult liver¹⁵. This study was designed to see the damaging effects of haloperidol on developing liver.

MATERIAL AND METHODS

45 female albino rats and 12 male albino rats of Sprague-drawly strain, weighing about 250-300 g were used. They were obtained from Pakistan Council of Scientific and Industrial Research (PCSIR), Karachi.

All animals male and females were kept separately in the animal house of the Punjab Postgraduate Medical Institute, Lahore and were acclimatized for 15 days. A twelve hour light and dark cycle was maintained at room temperature between 22-25°C. After acclimatization three female and one male rats were kept together in a cage for a week for conception and male rats were removed from the cage later on. Female rats were observed for vaginal plug (**Appendix II**)¹⁶. This was taken as day zero of pregnancy.

After conception female rats were randomly divided in three groups; A (control), B (experimental low dose) and C (experimental high dose), each group having 15 rats. Haloperidol in injectable form was given to the rats by intraperitoneal route (Fig-2) from 9th day of gestation onwards as liver primordium first appears on the 11th day of development¹⁷.

The dose schedule was as follows:

Control Group A:

It had 15 female rats which were given 0.2mg/kg body weight of phosphate-buffered saline intra

peritoneal from 9th to 21st day of gestation as a champ treatment.

Experimental Group B:

It had 15 female rats which were given 0.4mg/kg body weight of haloperidol intra peritoneal from 9th to 21st day of gestation.

Experimental Group C:

It had 15 female rats which were given 0.8mg/kg body weight of haloperidol intra peritoneal from 9th to 21st day of gestation.

These animals of each group (A,B and C) were then euthanized on 21st day of gestation by injecting sodium pentobarbital as anesthetic intraperitoneally in doses of 45mg/kg^{18,19} and morphine as analgesic in doses of 0.3-0.5mg/kg intraperitoneally²⁰. Hysterotomy was done and pups were removed. Average litter size of albino rats in each group was 5 per animal, so in this step of study the total number of the pups was 5 x 45 = 225. 24 pups from each group were randomly selected by lottery method (**appendix-III**)²¹. These pups were labeled as A1, B1, and C1.

Procedure of dissection:

The body weight of each animal was recorded before dissection, stretched out in supine position on the dissection tray. The limbs were fixed with the help of pins and a midline incision was given through the skin that extended from the xiphisternum to the pubic symphysis and abdominal wall was opened in the mid line with the help of scissors. The liver was identified, falciform and coronary ligaments were cut, common hepatic duct and hepatic vessels were incised and liver was dissected out. The liver was weighed and observed for any gross abnormality and preserved in 10% formalin for histological evaluation.

Measurement of size of Hepatocytes:

Under light microscopy, micrometry would be performed. In each slide 20 hepatocytes were selected randomly, size of each hepatocyte was measured with microscopic oculometer in transverse, anteroposterior and oblique dimensions. Average of these three dimensions was calculated to get average size of a hepatocyte. Then average of it was calculated. This average value was taken as final value.

Statistical analysis:

Data was analysed by SPSS version 21. Diameter of hepatocytes was described by Mean, ±S.D. and comparison between the groups were made by

ANOVA. The P-value less than 0.05 was considered as statistically significant.

RESULTS

The mean diameter of hepatocyte in control group was $13.85 \pm 0.71 \mu\text{m}$, while in group B1 it was recorded $12.07 \pm 0.95 \mu\text{m}$ and $12.04 \pm 1.45 \mu\text{m}$ in C1. The difference among three groups was statistically significant with p-value < 0.001 (Table-1)

Pair wise comparison yielded that the difference of groups B1 and C1 from A1 was both significant with p-value < 0.001 but the difference between group B1 and C1 was statistically insignificant with p-value 0.996. (Table-2, Fig-1)

Hepatic nucleus diameter in group A1 was $7.48 \pm 0.14 \mu\text{m}$ while in group B1 & C1 was $7.54 \pm 0.22 \mu\text{m}$ and $7.40 \pm 0.55 \mu\text{m}$ respectively (Table-3). The difference among three groups for hepatic nucleus diameter was statistically insignificant with p-value 0.357 (Table-4, Fig-2)

Groups	Mean	SD	Minimum	Maximum
Group A1	13.85	0.71	12.5	15.50
Group B1	12.07	0.95	10.0	13.75
Group C1	12.04	1.45	10.0	17.50

Table-1: Diameters of hepatocytes (μm) in control and experimental groups exposed to haloperidol

Key

- A1 Control Group
- B1 Experimental Group
- C1 Experimental Group
- SD Standard Deviation

	Sum of squares	Df	Mean square	F	P-value
Between Groups	51.82	2	25.91	22.21	< 0.001
Within Groups	80.48	69	1.17		
Total	132.3	71			

Table-2: Comparison of diameters of hepatocytes in control and experimental groups exposed to haloperidol

Groups	Group comparison	Mean difference	Std. error	P-value
Group A1	Group B1	1.79	0.31	< 0.001
	Group C1	1.81	0.31	< 0.001
Group B1	Group C1	0.03	0.31	0.996++

Table-3: Pair wise comparison of diameters of hepatocytes in control and experimental groups exposed to haloperidol

Key

- A1 Control group
- B1 Experimental group
- C1 Experimental group
- ** Highly significant difference (p-value < 0.001)
- ++ Insignificant difference

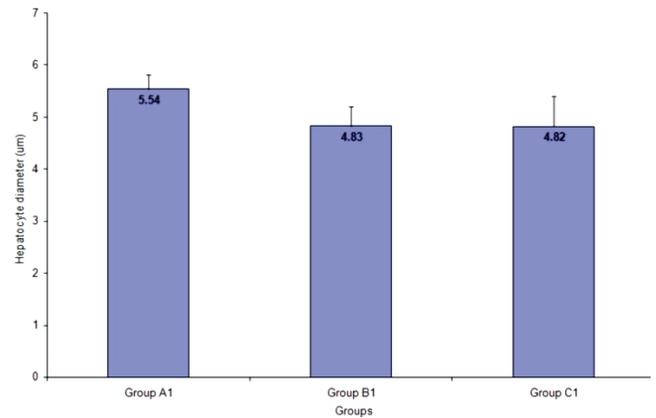


Fig-1: Graphic comparison of diameter of hepatocyte in pups of control group A1 and experimental groups B1 & C1 exposed to haloperidol

Groups	Mean	SD	Minimum	Maximum
Group A1	7.48	0.14	7.25	7.75
Group B1	7.54	0.22	7.25	8.25
Group C1	7.40	0.55	5.00	7.75

Table-4: Diameters of hepatocyte nucleus in the liver of control and experimental groups exposed to haloperidol

Key

- A1 Control group
- B1 Experimental group
- C1 Experimental group
- SD Standard deviation

	Sum of squares	Df	Mean square	F	P-value
Between Groups	0.26	2	0.13	1.05	0.357++
Within Groups	8.45	69	0.12		
Total	8.71	71			

Table-5: Comparison of diameter of hepatocyte nucleus in the liver of control and experimental groups exposed to haloperidol

Key

Df Degree of freedom

++ Insignificant difference

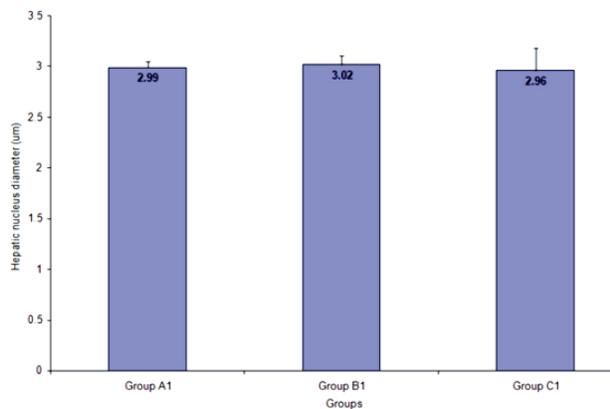


Fig-2: Comparison of diameter of hepatocyte nucleus in pups of control A1 and experimental groups B1 & C1 exposed to haloperidol

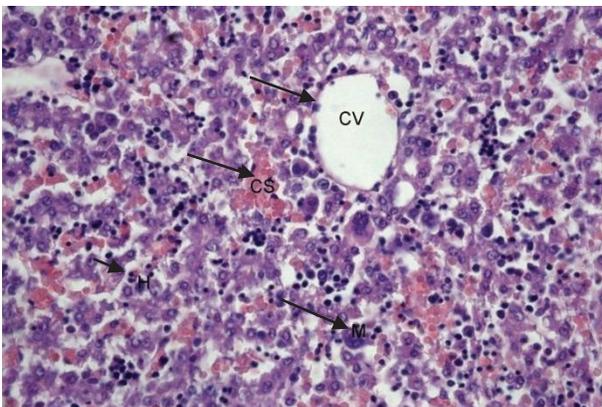


Fig-1: Photomicrograph of cross section of liver of pup of experimental group B1 showing, hepatocytes (H), centrilobular vein (CV), congested sinusoids (CS), multinucleated giant cells (M) (H&E, 40X)

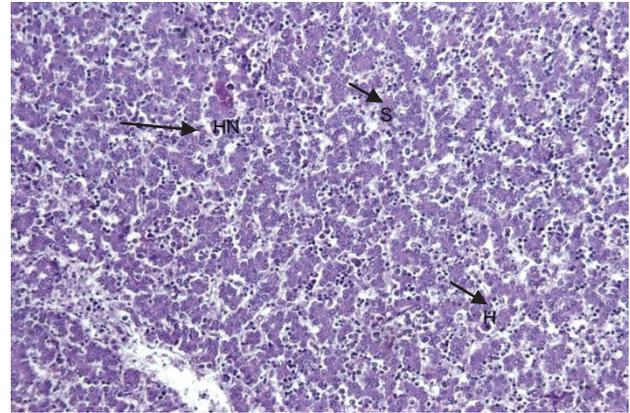


Fig-2: Photomicrograph of cross section of liver of pup of experimental group C1 showing hepatic nucleus (HN), hepatocytes (H), Sinusoids (S) (H&E, 20X)

DISCUSSION

In the present study the diameter of the hepatocytes and diameter of nucleus of the hepatocytes in experimental groups B1 and C1 were noted and compared with the control group A1. The results of this study revealed significantly decreased hepatocyte diameter in experimental groups B1 & C1 as compared with the control group A1 (p-value 0.001). Hepatic nuclear diameter, however seems to be unaffected in all the groups. The probable mechanisms for these reduced diameters are toxicity of haloperidol metabolites to liver cells resulting in cell atrophy and sequelae of cell adaptation to cell injury²². Injurious effects of haloperidol causes decreased synthesis of cellular binding blocks and increased breakdown of cellular organelles leading to atrophy of the cell. This may be due to the fact that haloperidol produces pyridine metabolites by cytochrome P450 mechanism which reduces glutathione inside the mitochondria and releases intracellular enzymes aminotransferases which are hepatic cell membrane injury markers²³. Depletion of glutathione results in oxidative stress with depletion of ATP and loss of oxidative phosphorylation. Reduced ATP results in failure of sodium pump with influx of sodium and water leading to cell swelling. Prolonged depletion of ATP due to persistent toxic stimuli leads to disruption of protein synthetic apparatus and the cells undergo necrosis²⁴. Necrosis is associated with loss of cell membrane integrity and leakage of cellular contents culminating in dissolution of cells. Nucleus become pyknotic characterized by nuclear shrinkage and increased basophilia. Atrophy in the liver cells and

pyknosis of nucleus suggest parenchymal injury leading to cell necrosis²⁵.

CONCLUSION

The present study revealed that haloperidol was responsible for destroying the normal architect of the developing liver. It was concluded that the liver growth was stunted as shown by poor development of hepatocytes. However, it is suggested that in spite of species differences, the antipsychotic drugs particularly haloperidol must not be given to pregnant women or must be stopped before pregnancy.

REFERENCES

1. What causes Psychosis [internet] 2006 Available from: <http://www.psychosissucks.ca/whatcausespsychosis.cfm>
2. XuB1, Ionita-LazaI, etal. De novo gene mutations highlight patterns of genetic and neural complexity in schizophrenia. *Nat Genet.* 2012; 44(12):1365–9.
3. Melmon KL Antipsychotic drugs. In: Melmon and Morelli's. *Clinical pharmacology.* New York: McGraw-Hill; 1999.
4. Haloperidol [internet] 2013 [updated 2014 May 14]. Available from: en.wikipedia.org/wiki/Haloperidol
5. Katzung BG. *Basic and clinical Pharmacology.* 10th ed. New York: Lange; 2007. p.479
6. Finnegan P, Buckingham R. Treatment of Acute Excited Psychosis with Intramuscular Haloperidol .*Can Fam Physician.* 1980; 26: 1199-1202.
7. Julia Hega di Labdani. Haloperidol [internet] 1992 [updated 1992 Sep 10]. Available from: <http://www.inchem.org/documents/pims/pharm/haloperi.htm>
8. Material safety data sheet 2007.
9. Patel AJ, Barochovsky O, Lewis PD. Psychotropic drugs and brain development: effects on cell replication in vivo and in vitro. *Neuropharmacology.* 1981; 20(12):1243-9.
10. Castro R, Brito B, and Notario V. Prenatal haloperidol alters the expression of DNA polymerases in brain regions of neonate rats. *Cell Mol Neurobiol.* 1990; 10(2): 281-289.
11. Williams R, Ali SF, Scalzo FM, Soliman K, Holson RR. Prenatal haloperidol exposure; effects on brain weights and caudate neurotransmitter levels in rats. *Brain Res Bull.* 1992; 29(3-4):449-58.
12. Mitchell J, Cooper AC, Griffiths MR, Cooper AJ. Acute administration of haloperidol induces apoptosis of neurons in the striatum and substantia nigra in the rat. *Neuroscience.* 2002; 109(1):89-99.
13. Gepdireme, N.Aydin , Halici Z, Sahin O, Unal, Aydin MD, Bakuridze K. Chronic treatment of haloperidol causes vasoconstriction on basilar arteries of rats, dose dependently. *Pharmacolo Res.* 2004; 50(6):569-74.
14. Halici Z, Dursun H, Keles O, Odaci E, Suleyman H, Ayden N, Cadirci E, Kalkan Y, Unal B. Effect of chronic treatment of haloperidol on the rat liver: a stereological and histopathological study. *Naunyn-Schmied Arch Pharmacol.* 2009; 379(3):253-61.
15. Obzek etall. Haloperidol induced Neuronal Damage in Guinea Pig Hippocampus. A Microscopic Study. *Journal of neurobiological sciences.*2010;27(4):438-445.
16. Saharrrm, Omar, Abed el samad. Modified vaginal smear cytology for the determination of the rat estrous cycle phases versus ordinary papanicolaou technique, verified by light and scanning electron microscopic examination of the endometrium .*The Egyptian journal of histology.* 2007; 30(2):397-408.
17. Laurence L, Brunton. *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 11th edition. NewYork: McGraw Hill Professional; 2005.
18. Lee-Parritz D. Analgesia for Rodent Experimental Surgery. *ISRAEL Journal of Veter Medi.* 2007; 62:3-4.
19. AVMA guidelines on Euthenasia.
20. British Society of Animal Sciences. Ethical guidelines for research in animal sciences. Jarvis S, Day J.E.L, Reed B.
21. Appendix III .Tutorial simple random sampling 2014 [internet] Available from: <http://www.emathzone.com/tutorials/basic-statistics/simple-random-sampling.html>
22. MohanH. *Textbook of pathology.* 6th edition. India: Jaypee Brothers Medical Publishers; 2005.

23. Anthony et al. Harrison's Principle of Internal medicine. 14th edition (2).New York: McGraw Hill; 1998.
24. Arthor S, Schneider MD, Philip A, Szanto MD. BRS Pathology. 5th edition. Lipponcott Williams & Wilkins; 2013.
25. Hangama M, Inoue H, Kamiya M, Schinone K, Nata M. Gene expression on liver toxicity induced by administration of haloperidol in rats with severe fatty liver. Legal Med. 2008; 10:177-184.

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