ISSN:2394-2371
CODEN (USA):IJPTIL

RESEARCH PAPER

Teratogenic and biochemical effects of Fluoxetine on Gallus gallus domesticus

Pinakin Wagh*, Navinya Gawali, Kaushik Karandikar
Operon Research and Learning, Kothrud, Pune, Maharashtra, India

*Corresponding Author: **Dr. Pinakin Wagh**

ABSTRACT

Fluoxetine is a commonly prescribed SSRI, used to treat neuropsychiatric disorders like depression, obsessive compulsive disorder and bulimia. This study was aimed at better understanding the teratogenic and biochemical effects of Fluoxetine on 4–5-day old Gallus gallus domesticus embryos (HH 24). A dose of 100ppm Fluoxetine administered in ovo showed teratogenic effects on the embryos. Biochemical analysis indicated a significant increase in the total protein content, slightly elevated Alkaline phosphatase levels and extremely diminished Acetylcholinesterase levels. This study provides insight into the effects of Fluoxetine and other SSRIs on gestating and lactating women as antidepressants pass through the placenta and breast milk, causing unwanted effects in the foetus.

Keywords: - *Fluoxetine, Gallus gallus domesticus, SSRI, Teratogenesis, Alkaline Phosphatase, Acetylcholinesterase.*

INTRODUCTION

Fluoxetine is a Selective Serotonin Reuptake Inhibitor (SSRI) and monoamine transmitter typically present in CNS. SSRIs are considered to be potent inhibitors of neuronal serotonin reuptake [1]. Fluoxetine is the most anorexic and stimulating SSRI [1]. It is an antidepressant that is used to treat neuropsychiatric symptoms like major depressive disorder, bulimia nervosa, bipolar I disorder, obsessive compulsive disorder, premenstrual dysphoric disorder (PMDD). Common side effects include anxiety, nausea, insomnia, heartburns, profuse sweating, fever, joint pain, shortness of breath, seizures, etc. Fluoxetine mainly targets the serotonergic synapse that serves as a target for physiological regulators. Fluoxetine can regulate plasticity in non-neurogenic regions [2].

Fluoxetine shows nonlinearity in pharmacokinetic profile [3]. Fluoxetine is a combination of R- and S-racemic mixture which coequally shows pharmacologically activity [4]. Saturation of metabolic pathways is observed due to non-linearity in pharmacokinetics. In contrast with its effect on other antidepressants, Fluoxetine has not been shown to change its pharmacodynamics depending on the age of the person [3]. Renal impairment and obesity

***CORRESPONDING AUTHOR**
Dr. Pinakin Wagh
Founder Director,
Operon Research and Learning Pune, India
E.Mail: pinakin76@gmail.com
Article Published: Jan. - March 2022
CITE THIS ARTICLE AS
Wagh P., Gawali N., Karandikar K. Teratogenic and biochemical effects of Fluoxetine on Gallus gallus domesticus. Int J Pharm Technol Biotechnol. 2022; 9(1):01-07.

are hardly affected by the pharmacodynamics of Fluoxetine [3]. Pharmacodynamics of Fluoxetine have shown no hazardous effects on diseases like Parkinson's disease; Fluoxetine shows the complementary effect on cognitive functions [4]. It is well understood that most antidepressants cross the placental barrier. The objective of the study was to better understand the effect of SSRIs on foetuses, as foetuses are especially susceptible towards teratogens during first trimester of pregnancy. *Gallus gallus domesticus* is a scientific model organism that plays a vital role in the fields of developmental biology, molecular biology, genetics, cell biology and many more. *G. domesticus* has a well-distinguished body plan and it is also amenable to genetic and physiological manipulations [6]. *G. domesticus* shows 70% functional protein orthologs with humans [6], making chick embryos dependable organisms to study. To study the effects of Fluoxetine on early embryonic development we used 4-5d old (HH 24) *G. domesticus* embryos. Fluoxetine has shown intricate gene interaction with genes like SLC6A2, SLC6A4, 5HTR1A, 5HTR2A, 5HTR2B, 5HTR2C, NR3C1, in *G. domesticus*[5].

MATERIALS AND METHODS

Chick embryo culture and dose response studies

Fertilized eggs of 4-5 d old chick embryos (HH 24) [7] of *Gallus gallus domesticus* (White Leghorn strain) were procured from Venkateshwara Hatcheries Pvt Ltd, Pune. Eggs were cleaned with distilled water and wiped with disinfectant to ensure sterility. Freshly prepared 100ppm Fluoxetine (FLUNIL, INTAS Pharmaceuticals Pvt. Ltd. 4mg/ml) was administered *in ovo* by air sac route. Control and treated embryos were incubated for 24 hours at 37.8°C with relative humidity of 70-80% in a BOD incubator (Bio-technics India). Post treatment, embryos were harvested in sterile chilled 1X PBS pH 7.4 and then observed for drug-induced anomalies by morphological observations under stereo zoom binocular microscope (MagnusTM)

Biochemical studies

Control and treated embryos were homogenized (Potter-Elvehjem PTFE pestle). Embryo homogenates were prepared using sterile 1X PEB. To study protein quantification in the samples, Bradford assay of protein estimation [8] was used. Bradford's reagent (ML106, HiMedia) was added to the homogenized samples and they were incubated for 20 mins. Optical density was measured (Systronics µC colorimeter 115) at absorbance 595 nm for control and treated homogenized sample. Bovine serum albumin was taken as a standard to estimate the protein content of the samples. Enzyme assays for Alkaline Phosphatase (ALP) and Acetylcholinesterase (AChE) were conducted. The ALP assay was performed using UltiChem- Alkaline phosphatase kit, Yucca diagnostics and AChE assay was performed using Delta Cholinesterase, Delta lab. Butyrl Thiocholine was used as substrate for both enzyme assays. Both the assays were analyzed using HORIBA Yumizen, CA60 semi-automatic analyzer.

RESULTS

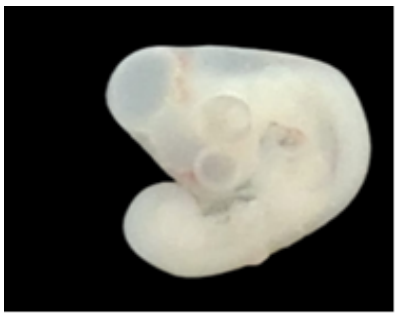

Fig.1 A: 4-5d control embryo

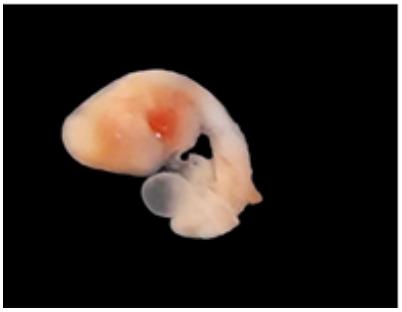

Fig.1 B: 4-5d 100ppm Fluoxetine treated embryo

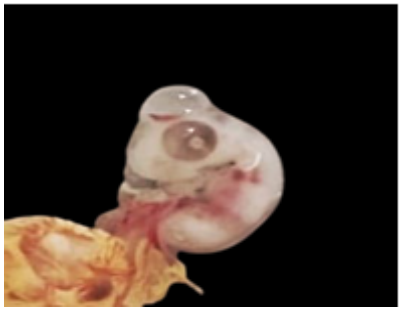

Fig.1 C: 4-5d 100ppm Fluoxetine treated embryo

Bradford assay of protein estimation

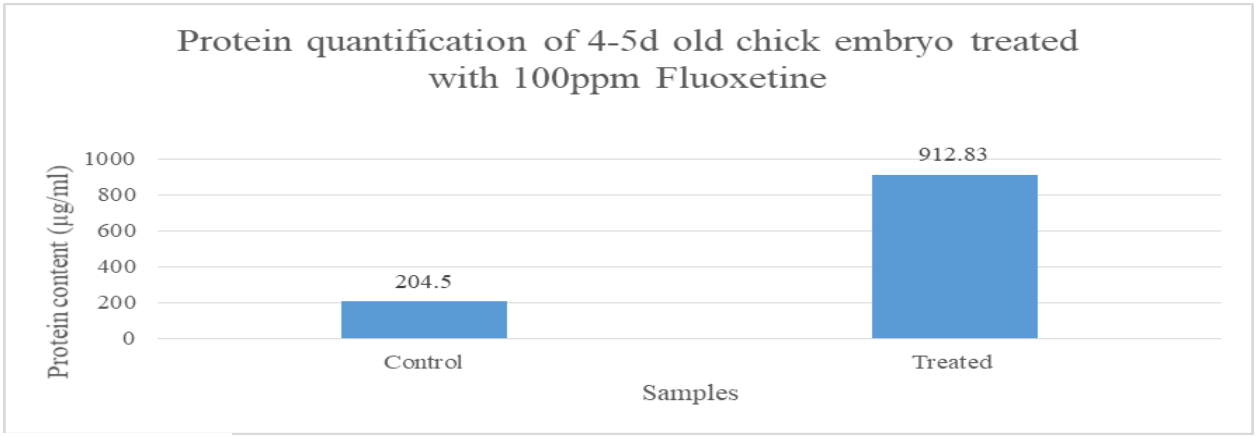

Figure 2- Protein quantification performed with the help of Bradford assay on 4-5d old chick embryos treated with 100ppm Fluoxetine

Alkaline Phosphatase (ALP)

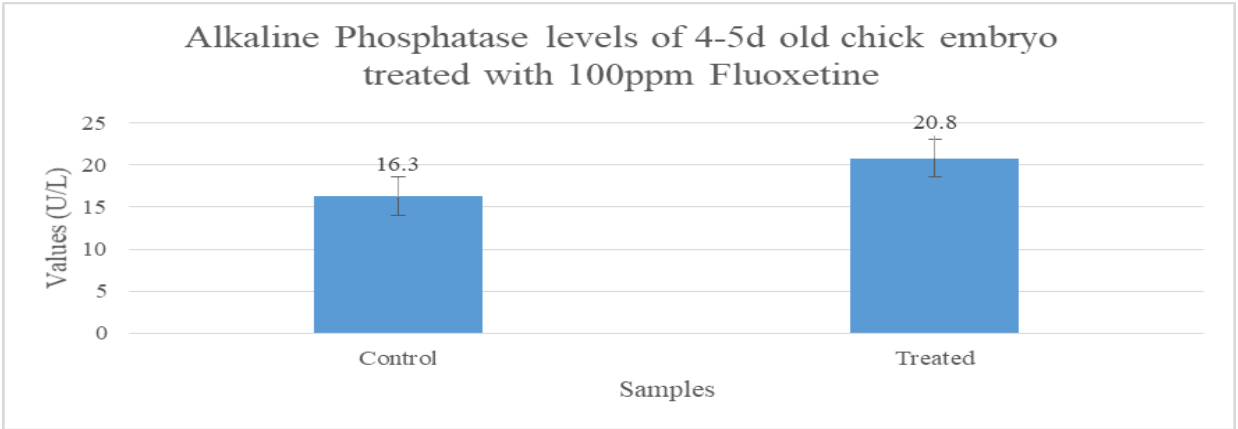

Figure 3- Alkaline Phosphatase enzyme assay performed on 4-5d old chick embryo treated with 100 ppm Fluoxetine.

Acetylcholinesterase (AChE) assay

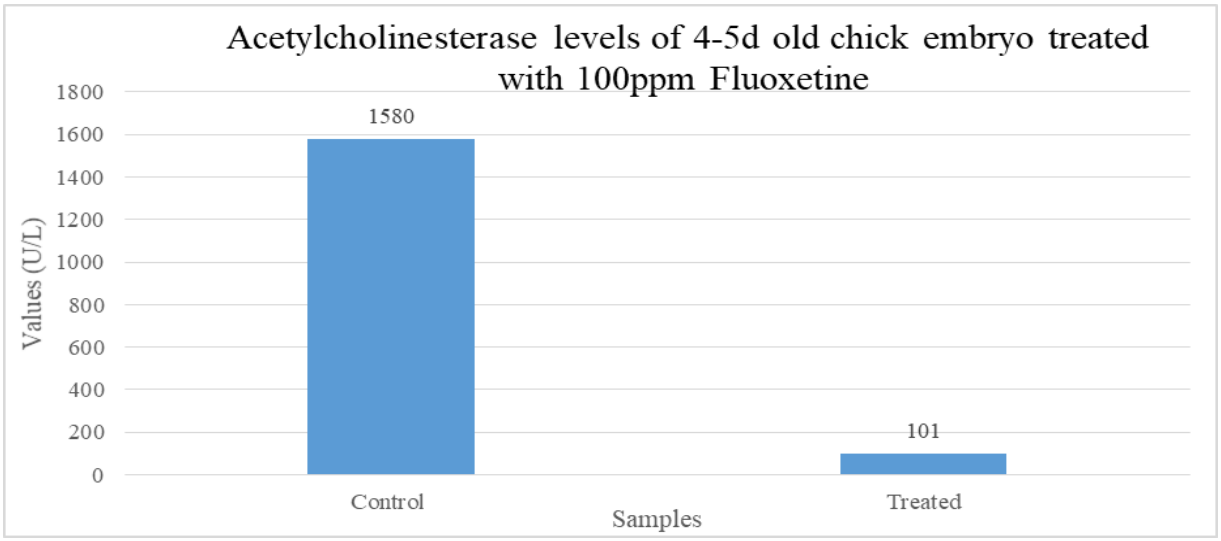

Figure 4- Acetylcholinesterase enzyme assay performed on 4-5 d old chick embryo treated with 100ppm Fluoxetine.

DISCUSSION

The present study shows that treatment of 4-5d old chick embryos with the antidepressant drug Fluoxetine induced teratogenesis. Post-treatment analysis of treated embryos showed morphological as well as chemical changes. Embryos treated with Fluoxetine had enlarged brain, enlarged optic cups and abnormal torsion. Some treated embryos showed ocular defects and abnormal morphology (Fig.1 B, C). From our results, we can infer that Fluoxetine has a teratogenic effect on 4-5d old *G. domesticus* embryos. Exposure to Fluoxetine during the first trimester of pregnancy in humans has shown a potential association with increased risk of cardiovascular anomalies [10]. However, a meta-analytical study showed no significant teratogenic effects during the first trimester during human pregnancy [11]. Fluoxetine has shown adverse effects on the viability of embryonic stem cells and embryonic fibroblasts of mice [12]. In rats, prenatal administration of high dose Fluoxetine showed a transient delay in motor development but led to an increase in memory and learning [13]. From our study, we conclude that Fluoxetine shows a gradual increase in the ALP levels in *G. domesticus.* In a study performed on mice osteoblasts, Fluoxetine showed an inhibitory effect on osteoblast differentiation, mineralization, and proliferation leading to elevation in ALP levels [14]. In a clinical study based on two cases of hepatitis induced by chronic use of Fluoxetine, elevated ALP levels were observed [15]. Fluoxetine treatment in depressed women with advanced cancer showed increased ALP levels [16]. However, studies on the safety of Fluoxetine in children and adolescents with major depressive disorder showed a slight decrease in ALP levels within normal limits [17]. In contradiction

to our results, a decrease in expression of ALP was seen in human adipose-derived stem cells treated with Fluoxetine [18].

Our results show an extreme decrease in AChE level on 4-5d chick embryos treated with Fluoxetine. In an experiment performed on zebrafish, it was concluded that Fluoxetine inhibits developmental and neurotoxic effects while it shows no effect in AChE level [19]. A pharmacological study performed on olfactory bulbectomized mice showed an increase in hippocampal AChE levels when treated with Fluoxetine [20]. However, literature on the effect of Fluoxetine on male mice reported no significant change in AChE levels [21]. Protein quantification of Fluoxetine treated embryos showed a significant rise as compared to control embryos; 346.37% increase was seen in the total protein content of treated samples. However, treatment of Fluoxetine on *Daphnia magna* showed a decrease in total protein content [22]. A study on HT29 and Caco-2 human colorectal adenocarcinoma cell lines also indicated a reduction in levels of total protein content [23].

ACKNOWLEDGEMENTS

We would like to extend our gratitude towards Operon research and learning, Pune for letting us conduct our research study. We would also like to thank our colleagues, Shravani Maral and Hrithika Shinde for assisting us throughout our project.

CONCLUSION

Fluoxetine has demonstrated teratogenic effects in 4-5d old *G. domesticus* embryos at the concentration 100ppm. It also increased total protein content, slightly increased Alkaline phosphatase levels, and significantly decreased Acetylcholinesterase levels. These results can be extrapolated onto effect of Fluoxetine on gestating and lactating women.

REFERENCES

1. Szklarczyk.D, fluoxetine (Homo sapiens), STITCH consortium 2016, version 5.0, http://stitch.embl.de/cgi/network.pl?taskId=BqDriO4MJbnA
2. Maya Vetencourt JF, Sale A, Viegi A, et al. The antidepressant Fluoxetine restores plasticity in the adult visual cortex. *Science*. 2008;320(5874):385-388. doi:10.1126/science.1150516
3. Altamura AC, Moro AR, Percudani M. Clinical pharmacokinetics of Fluoxetine. *Clin Pharmacokinet*. 1994;26(3):201-214. doi:10.2165/00003088-199426030-00004
4. Harris, M.G., Benfield, P. Fluoxetine. *Drugs & Aging* **6,** 64–84 (1995). https://doi.org/10.2165/00002512-199506010-00006
5. Szklarczyk.D, fluoxetine (Gallus gallus), STITCH consortium 2016, version 5.0, http://stitch.embl.de/cgi/network.pl?taskId=NpJ9tVSMM3fT

6. Vilches-Moure JG. Embryonic Chicken (*G. domesticus domesticus*) as a Model of Cardiac Biology and Development. *Comp Med*. 2019;69(3):184-203. doi:10.30802/AALAS-CM-18-000061
7. International Chicken Genome Sequencing Consortium. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432,** 695–716 (2004). https://doi.org/10.1038/nature03154
8. Hamburger, V., & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *Journal of morphology*, *88*(1), 49-92.
9. Kruger, N. J. (2009). The Bradford method for protein quantitation. *The protein protocols handbook*, 17-24.
10. Diav-Citrin, O., Shechtman, S., Weinbaum, D., Wajnberg, R., Avgil, M., Di Gianantonio, E., ... & Ornoy, A. (2008). Paroxetine and Fluoxetine in pregnancy: a prospective, multicentre, controlled, observational study. *British journal of clinical pharmacology*, *66*(5), 695-705.
11. ADDIS, A., & KOREN, G. (2000). Safety of Fluoxetine during the first trimester of pregnancy: a meta-analytical review of epidemiological studies. *Psychological medicine*, *30*(1), 89-94.
12. Kusakawa, S., Yamauchi, J., Miyamoto, Y., Sanbe, A., & Tanoue, A. (2008). Estimation of embryotoxic effect of Fluoxetine using embryonic stem cell differentiation system. *Life sciences*, *83*(25-26), 871-877.
13. Bairy, K. L., Madhyastha, S., Ashok, K. P., Bairy, I., & Malini, S. (2007). Developmental and behavioral consequences of prenatal Fluoxetine. *Pharmacology*, *79*(1), 1-11.
14. Bradaschia-Correa, V., Josephson, A. M., Mehta, D., Mizrahi, M., Neibart, S. S., Liu, C., ... & Leucht, P. (2017). The selective serotonin reuptake inhibitor Fluoxetine directly inhibits osteoblast differentiation and mineralization during fracture healing in mice. *Journal of Bone and Mineral Research*, *32*(4), 821-833.
15. Cai, Q., Benson, M. A., Talbot, T. J., Devadas, G., Swanson, H. J., Olson, J. L., & Kirchner, J. P. (1999, July). Acute hepatitis due to Fluoxetine therapy. In *Mayo Clinic Proceedings* (Vol. 74, No. 7, pp. 692-694). Elsevier.
16. Holland, J. C., Romano, S. J., Heiligenstein, J. H., Tepner, R. G., & Wilson, M. G. (1998). A controlled trial of Fluoxetine and desipramine in depressed women with advanced cancer. *Psycho-Oncology: Journal of the Psychological, Social and Behavioral Dimensions of Cancer*, *7*(4), 291-300.
17. Nilsson, M., Joliat, M. J., Miner, C. M., Brown, E. B., & Heiligenstein, J. H. (2004). Safety of subchronic treatment with Fluoxetine for major depressive disorder in children and adolescents. *Journal of Child & Adolescent Psychopharmacology*, *14*(3), 412-417.

18. Khademi, M., Taghizadeh Ghavamabadi, R., Taghavi, M. M., Shabanizadeh, A., Shariati-kohbanani, M., Hassanipour, M., & Taghipour, Z. (2019). The effects of Fluoxetine on the human adipose-derived stem cell proliferation and differentiation. *Fundamental & clinical pharmacology*, *33*(3), 286-295.
19. de Farias, N. O., Oliveira, R., Sousa-Moura, D., de Oliveira, R. C. S., Rodrigues, M. A. C., Andrade, T. S., ... & Grisolia, C. K. (2019). Exposure to low concentration of Fluoxetine affects development, behaviour and acetylcholinesterase activity of zebrafish embryos. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *215*, 1-8.
20. Machado, D. G., Cunha, M. P., Neis, V. B., Balen, G. O., Colla, A., Grando, J., ... & Rodrigues, A. L. S. (2012). Fluoxetine reverses depressive-like behaviors and increases hippocampal acetylcholinesterase activity induced by olfactory bulbectomy. *Pharmacology Biochemistry and Behavior*, *103*(2), 220-229.
21. McCloskey, M. C., Young, T. J., & Anderson, S. M. (2017). The influence of acetylcholinesterase on anxiety-and depression-like behaviors in Fluoxetine-treated male mice. *Bios*, *88*(1), 29-38.
22. Över, S. B., Güven, C., Taskin, E., & Sevgiler, Y. (2020). Oxidative and apoptotic effects of Fluoxetine and its metabolite norFluoxetine in Daphnia magna. *Arhiv za higijenu rada i toksikologiju*, *71*(3), 211-221
23. Kannen, V., Garcia, S. B., Silva Jr, W. A., Gasser, M., Moench, R., Alho, E. J. L., ... & Stopper, H. (2015). Oncostatic effects of Fluoxetine in experimental colon cancer models. *Cellular signalling*, *27*(9), 1781-1788..