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Prenatal exposure to the fluoride containing psychiatric drug fluoxetine and anti-oxidative alterations in the neonatal rat brain

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Abstract: Fluoride is a key ingredient of many psychiatric drugs like fluoxetine (Prozac®, Fluoxetine®). Pregnant women frequently use this drug as they suffer from depression and anxiety disorders during this period. Fluoxetine is able to reach the fetus through the placenta and passes to the newborn through milk. In the present study, female Wistar rats were treated with 5, 10, and 20 mg/L fluoxetine (containing 94% fluorides) from pregnancy day 10 to day 20. After delivery, the levels of the enzymatic antioxidants in the brain of their offspring at postnatal day 2 were measured. The results showed that, in all fluoxetine exposed groups compared with the control group, there was a significant decrease ($P < 0.01$) in the glutathione, catalase, glutathione S-transferases and potassium and a non- significant increase ($P > 0.05$) in the activity of malondialdehyde and creatine kinase. The results suggest that fluoxetine may be a developmental neurotoxicant due to presence of fluoride hence must be used carefully during pregnancy.

Introduction

Most of the frequently used fluorides containing drugs are antidepressants, anti-inflammatory, anti-anxiety, antimalarial, antibiotics, steroids, and cholesterol-lowering agents. In addition to these, chemotherapeutic agents for cancer treatment also contain fluorine (Strunecká *et al.*, 2004). Substitution of fluorine in a particular drug can affect its pharmacokinetic pharmacodynamics properties, especially its absorption, route and rate of biotransformation and, above all, its toxicology (Park *et al.,* 2001). Studies have also found that fluoride-containing drugs can increase the fluorinated compounds in serum and urine of rats (Henry *et al.,* 2005; Thompson *et al.,* 2000) and can pass through the placental and blood brain barriers (Fleschler *et al.,* 2008).

Prescription of antidepressant drugs, particularly fluoxetine, which contain 94% fluorides, has increased during pregnancy and/or lactation in women (Thompson *et al.*, 2000). Fluoxetine belongs to the pharmacological group of the serotonin reuptake selective inhibitors. Fluoxetine cross the human placenta and its active metabolites are excreted into milk. Intake of this drug does not influence the physiological levels of fluoride in serum but 4-trifluoromethylphenol which is one of its metabolite is able to increase fluoride ion in a time and concentration-dependent manner in humans (Fleschler *et al.,* 2008; Karadeniz and Altintas, 2008). L.4-trifluoromethylphenol is known to be cytotoxic and is metabolized into hydrogen fluoride under physiological conditions (Henry *et al.,* 2005; Thompson *et al.,* 2000). Fluoride crosses the cell membranes and affects several tissues, including the blood, and the brain (Shashi, 2003; Flora *et al.*, 2009). Several reports have shown that fluoride can cause lipid peroxidation and modify the levels of antioxidant enzymes in the brain (Vani and Reddy, 2000) Also, fluoride can accumulate in the brain, leading to abnormal behavioral patterns, impairment of cerebrovascular integrity and neuronal function, as well as causing metabolic lesions (Bayne *et al.*, 2010). In this general context, the present study investigates the possible depletion of antioxidants in the brain tissue of rat pups exposed in utero to fluorine-containing drugs such as fluoxetine.

Material and Methods

Animals

Adult Wistar albino rats of both sexes, weighing approximately

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hundred grams body weight were purchased from the Animal House of Science College, King Saud University, Riyadh. The animals were kept in cages, with wood shavings as bedding material, and were fed with a standard pelleted diet, and maintained at 21 ± 1°C room temperature, and a 12-12 h light-dark cycle. After one week, male and female rats were mated overnight. The occurrence of a vaginal plug was checked in the morning, thus determining pregnancy day 0. All the pregnant rats were kept in individual cages and randomly divided into four groups. The control group was given distilled water, the remaining three groups were exposed orally to 5, 10, 20 mg/kg body wt. of fluoxetine from pregnancy day 10 to day 20. The protocols used in the present study were approved by the Ethics Committee at the King Saud University.

Brain sampling and preparation

Two days after delivery, the rat pups were anesthetized using carbon dioxide and killed by decapitation. Then the whole brain was dissected out and washed with normal saline. Afterwards, it was homogenized in bi-distilled water (10 times w/v). Finally, the homogenate was centrifuged at 3000 × g for 15 min at 4°C . The clear supernatant was collected and used for biochemical assays.

Biochemical analyses

Lipid oxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARS), according to Ruiz-Larrea *et al.,* 1994. Glutathione was determined by the method of Beutler *et al.,* 1963, which uses 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) and sulfhydryl compounds to produce a yellow compound. Glutathione S-transferase (GST) activity was determined according to Habig *et al.*, 1974; this assay is based on the GST-catalyzed reaction between glutathione, and CDNB (1-chloro-2, 4-dinitrobenzene).

TABLE 1

Mean ± S.D. of all the measured parameters in all groups

Parameter	Group	(Fluoxetine, mg/kg)	N	Mean ± S.D.	Percent change	*P* value[a]	*P* value[b]
Lipid peroxides (MD µmoles/ml)	Control	(0 mg/kg)	14	0.33 ± 0.08	100.00		0.113
	Group I	(5 mg/kg)	18	0.34 ± 0.05	102.17	0.781	
	Group II	(10 mg/kg)	15	0.36 ± 0.05	111.22	0.176	
	Group III	(20 mg/kg)	17	0.37 ± 0.05	113.21	0.103	
Glutathione (ug/ml)	Control	(0 mg/kg)	14	36.97 ± 8.87	100.00		0.001
	Group I	(5 mg/kg)	18	19.08 ± 6.75	51.62	0.001	
	Group II	(10 mg/kg)	15	16.39 ± 4.40	44.34	0.001	
	Group III	(20 mg/kg)	17	23.67 ± 3.77	64.02	0.001	
Catalase (U/dl)	Control	(0 mg/kg)	14	6.36 ± 1.45	100.00		0.011
	Group I	(5 mg/kg))	18	3.08 ± 1.15	48.50	0.001	
	Group II	(10 mg/kg)	15	4.87 ± 2.02	76.56	0.032	
	Group III	(20 mg/kg)	17	4.94 ± 1.83	77.69	0.026	
Glutathione S-transferases activity (U/ml)	Control	(0mg/kg)	14	15.54 ± 6.23	100.00		0.001
	Group I	(5 mg/kg)	18	6.76 ± 2.19	43.51	0.001	
	Group II	(10 mg/kg)	15	8.51 ± 2.86	54.73	0.001	
	Group III	(20 mg/kg)	17	19.29 ± 9.93	124.14	0.230	
Potassium (mmol/L)	Control	(0 mg/kg)	14	5.64 ± 1.69	100.00		0.001
	Group I	(5 mg/kg)	18	2.36 ± 1.84	41.76	0.001	
	Group II	(10 mg/kg)	15	2.96 ± 1.13	52.48	0.001	
	Group III	(20 mg/kg)	17	2.52 ± 1.39	44.76	0.001	
Creatine Kinase (IU/L at 30 degree C)	Control	(0 mg/kg)	14	283.69 ± 81.14	100.00		0.210
	Group I	(5 mg/kg)	18	340.67 ± 30.25	120.08	0.098	
	Group II	(10 mg/kg)	15	331.50 ± 49.45	116.85	0.158	
	Group III	(20 mg/kg)	17	339.08 ± 65.43	119.52	0.140	

[a] *P* value between control group and other groups.

[b] *P* value between all groups.

TABLE 2

Pearson's correlations between the measured parameters

Parameters	R (Pearson correlation)	Sig.	
Lipid peroxides (MD µmoles/ml) ~ creatine kinase (IU/L at 30°C)	0.392*	0.024	P[a]
Glutathione (ug/ml) ~ catalase (U/dl)	0.375**	0.002	P[a]
Glutathione (ug/ml) ~ GST activity (U/ml)	0.422**	0.001	P[a]
Glutathione (ug/ml) ~ potassium (mmol/L)	0.459**	0.001	P[a]
Catalase (U/dl) ~ GST activity (U/ml)	0.323**	0.009	P[a]
Potassium (mmol/L) ~ creatine kinase (IU/L at 30°C)	-0.354*	0.043	N[b]

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.
[a] Positive correlation
[b] Negative correlation

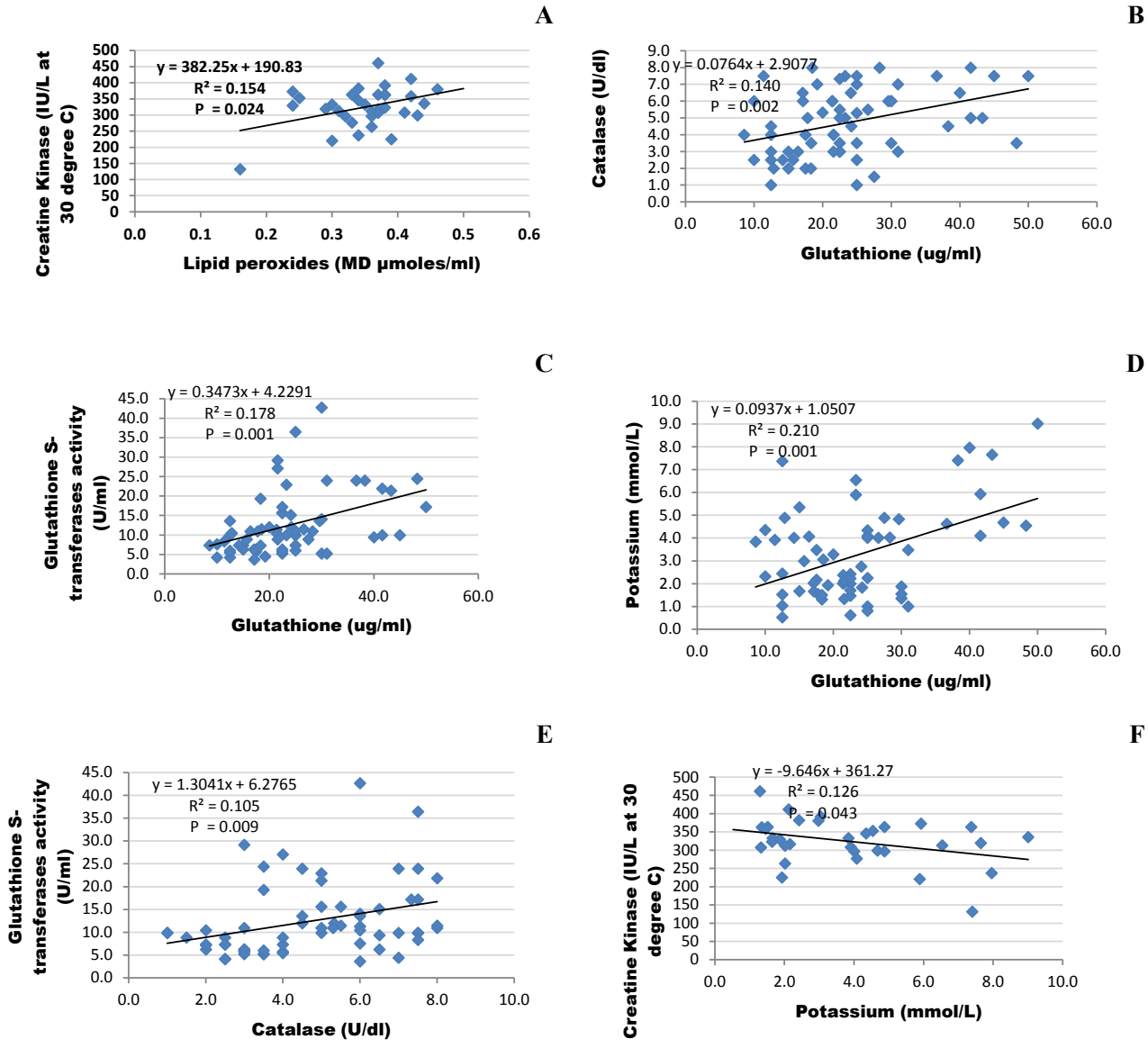

FIGURE 1. Correlation between A-Lipid peroxides (MD µmoles/ml) and creatine kinase (IU/L at 30 degree C); B-Glutathione (ug/ml) and catalase (U/dl); C-Glutathione (ug/ml) and glutathione S-transferases; D-Glutathione (ug/ml) and potassium (mmol/L); E-Catalase (U/dl) and glutathione S-transferases activity (U/ml) (positive correlation), F-Potassium (mmol/L) and creatine kinase (IU/L at 30 degree C)(negative correlation) with best fit line curve.

Catalase was measured according to the method of Maehly and Chance (1954) in which the rate of hydrogen peroxide (H_2O_2) dissociation per minute by the enzyme was followed by measuring the change in absorbance at 240 nm.

The method of Terri and Sesin (1958) was used to estimate potassium levels in which turbidity is produced by a reaction with sodium tetraphenyl boron in a protein-free alkaline medium.

Creatine kinase was determined by the method of Szasz (1955), using a kit purchased from NSC Human, Germany.

Statistical analyses

All the analysis was done by using SPSS, Chicago, IL, USA. The results are presented as mean ± standard deviation (S.D). One-way analysis of variance (ANOVA) was complemented with the Dunnett test for multiple comparisons. The significance level was fixed at $P < 0.05$. Receiver operating characteristics curve (ROC) and Pearson's correlations were also performed, when appropriate.

Results

The brain malondialdehyde concentrations were found elevated in rat pups exposed to fluoxetine during gestation, at several concentrations, however the increases were not statistically significant. Fluoxetine, at concentrations 5, 10 and 20 mg/kg body weight caused statistically significant decreases ($P < 0.001$) in reduced glutathione levels in the brain, as compared with that of control rat pups, by 52, 44 and 64%, respectively. Fluoxetine, at concentrations of 5 and 10 mg/kg body weight caused a caused a significant decrease ($P < 0.001$) in the activity of brain glutathione S-transferase by 44 and 54%, respectively, when compared to control rat pups. Fluoxetine however, at a concentration of 20 mg/kg body weight, had no significant effect on brain glutathione S-transferase activity when compared to control rat pups ($P > 0.05$). Fluoxetine at concentrations 5, 10 and 20 mg/kg body weight caused a significant inhibition of catalase in the brain, by 50, 77 and 78%, respectively, when compared with control rat pups ($P < 0.05$). Creatine kinase showed no significant increases in rat pups which were exposed to the different concentrations of fluoxetine (Tab. 1).

TABLE 3

ROC-Curve of all parameters in all groups

	Group	Area under the curve	Best Cutoff value	Sensitivity %	Specificity %
Lipid peroxides (MD μmoles/ml)	Group I	0.614	0.355	61.1 %	60.9 %
	Group II	0.597	0.335	80.0 %	42.9 %
	Group III	0.608	0.305	100.0 %	29.8 %
Glutathione (ug/ml)	Group I	0.673	17.650	55.6 %	80.4 %
	Group II	0.822	21.510	93.3 %	73.5 %
	Group III	0.586	18.050	100.0 %	42.6 %
Catalase (U/dl)	Group I	0.830	4.750	94.4 %	67.4 %
	Group II	0.522	5.750	46.7 %	67.3 %
	Group III	0.551	4.750	64.7 %	55.3 %
Glutathione S-transferases activity (U/ml)	Group I	0.873	7.940	83.3 %	84.8 %
	Group II	0.651	11.960	100.0 %	42.9 %
	Group III	0.832	11.960	76.5 %	83.0 %
Potassium (mmol/L)	Group I	0.705	2.595	77.8 %	60.9 %
	Group II	0.511	4.445	93.3 %	28.6 %
	Group III	0.648	3.655	82.4 %	48.9 %
Creatine Kinase (IU/L at 30 degree C)	Group I	0.604	314.750	85.7 %	46.2 %
	Group II	0.572	307.800	88.9 %	37.5 %
	Group III	0.502	402.100	22.2 %	100.0 %

Tab. 2 and Fig. 1 present Pearson's correlations between the measured parameters. Lipid peroxide was positively correlated with creatine kinase (Pearsons R = 0.392; P = 0.024). Glutathione was positively correlated with GST, potassium and catalase (Pearsons R = 0.422; P = 0.001), (Pearsons R = 0.459; P = 0.001) and (Pearsons R = 0.375 P = 0.002) respectively. Also catalase (U/dl) was positively correlated with glutathione S-transferases (Pearsons R = 0.323 P = 0.009). In addition Potassium was negatively correlated with creatine kinase (Pearsons R = - 0.354 P = 0.043).

Tab. 3 and Fig. 2 show the ROC analysis with the area under the curve (AUC), specificity and sensitivity of the measured parameters under various experimental conditions showing the probability of using glutathione, GST, lipid peroxides, creatine kinase and potassium as markers of fluoxetine neurotoxicity.

Discussion

A significant number of infants are exposed to fluoride containing antidepressant medications prior to birth or while nursing**.** Overall, over 5% of pregnant women use fluoxetine containing 94% fluoride during pregnancy (Andrade *et al.*, 2008). As in humans, fluoxetine crosses the placental and fetal blood-brain barriers in rats and mice (Olivier *et al.*, 2011). Therefore, these animals can serve as useful models for investigation of parameters that would not easily be probed in humans, particularly oxidative stress in brain tissue of infants. The present study examined the dose dependent effect of fluoxetine on oxidative stress in brain of new born rats exposed in utero for ten days.

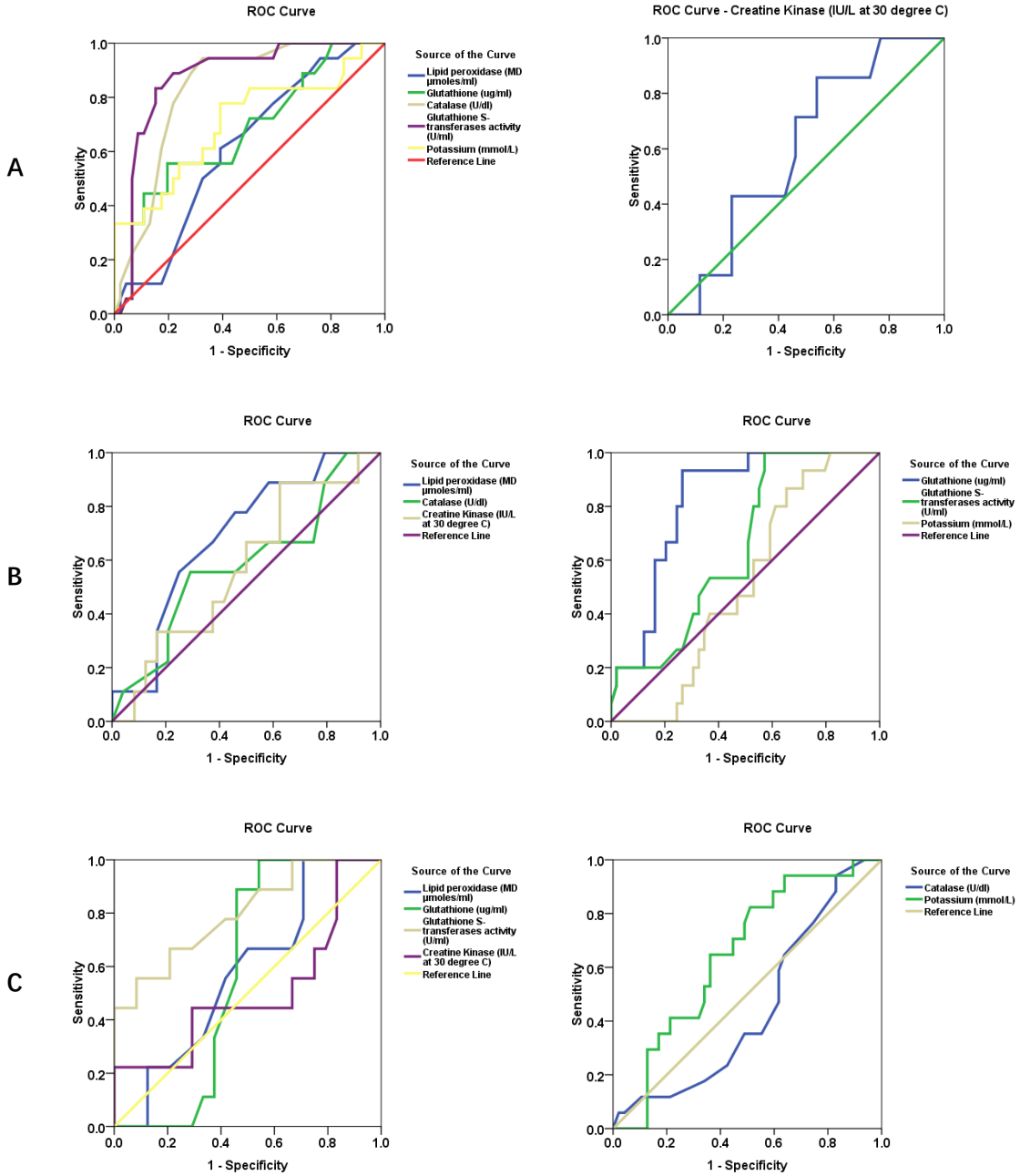

FIGURE 2. ROC Curve of all parameters in A- Group I, B- Group II, C- Group III.

The new-borns exposure to fluoxetine during the uterine period showed significant decrease in glutathione, and anti-oxidant enzymes like catalase and glutathione S-transferases. It has been reported that 4-trifluoromethylphenol, one of the metabolites of fluoxetine decreases intracellular anti-oxidant enzymes levels (Ahmed *et al.,* 2014). The reduction in the levels of reduced glutathione in fluoxetine exposed infants brain may be due to their contribution to the antioxidant effects in neutralizing the free radicals (Bilici *et al.,* 2001). Further may also be attributed to the probable decrease in the activity of glutathione S-transferases. Antioxidant enzymes are reported to be decreased in the serum of fluoxetine treated rats (Adzic *et al.,* 2011). However abrupt increase of glutathione S-transferases was found at 20 mg/kg dose of fluoxetine in Group III (Tab. 1) this increased glutathione S-transferases due to high dose of fluoxetine exposure could be a counteracting mechanism adopted to eliminate fluoxetine. In this study, lipid peroxidation, as measured by TBARS, an index of malondialdehyde production. was found to be non- significantly elevated in the brain tissue groups of fluoxetine -treated rats, as compared to the control group. This may indicate low oxidative stress generation or neutralization of free radicals by the antioxidant defense enzymes in the brain. Moreover, lipid peroxidation mediated through free radicals can result in severe tissue damage and membrane disorganization resulting in decreased membrane fluidity.

The increase of brain creatine kinase reported in the present study can be related to the effect of fluoxetine on serotonin transporters. Direct action of liver bio accumulated fluoxetine on liver metabolism through modulation of serotonergic input cannot be ignored (Ramirez *et al.,* 2009). The increase of brain creatine kinase in fluoxetine -treated rats can be easily related to the recent study of Polakof *et al.,* 2007 in which they reported that a dose-dependent decrease in food intake, weight gain, glucose metabolism and glycolytic activity in fluoxetine-treated fish and to Mennigen *et al.,* 2010 who confirm brain gene expression patterns in line with potential anorexigenic effects of fluoxetine in the hypothalamus, with increased expression in corticotropin-releasing factor (CRF) and decreased expression of neuropeptide Y (NPY) in fluoxetine-treated fish. Creatine kinase as energy generating enzyme can be activated to compensate and replenish ATP depletion that might be occurred in response to fluoxetine treatment. The significant decrease in potassium reported in all fluoxetine treated infants may be due to cytotoxic effect of 4-trifluoromethylphenol which is one of the active metabolite of the drug. Cytotoxicity of 4-trifluoromethylphenol due to the loss of intracellular potassium has been reported earlier (Fleschler *et al.,* 2008)

The positive correlations of glutathione (as a marker of oxidative stress) with catalase, glutathione S-transferases and potassium and negative correlation of potassium with creatine kinase (Tab. 2, Fig. 1) showed neurotoxicity of fluoxetine by oxidative stress among the etiological mechanism. Also AUC, the specificity and sensitivity values listed in Tab. 3 demonstrate the possibility of using glutathione, glutathione S-transferases, Lipid peroxides and potassium as markers of fluoxetine neurotoxicity at 10 mg/kg and 20 mg/kg dose in Group I and Group II. However at low dose of 5 mg/kg these parameters appears as poor markers of fluoxetine neurotoxicity (Fig. 2)

In conclusion, fluoxetine exposure to mother's rats exert effects on pups brain through aggravated oxidative stress and cell damage. Hence its use during pregnancy must be considered when its benefits outweigh its toxic effects. Also unnecessary use of higher doses of the drug during pregnancy should be avoided.

Conflict of Interests

The authors declared no conflict of interests.

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References

Adzic M, Djordjevic J, Mitic M, Simic I, Rackov G, Djordjevic A, Elakovic I, Gordana M, Radojcic M (2011). Fluoxetine decreases glutathione reductase in erythrocytes of chronically isolated wistar rats. *Acta Chimica Slovenica* **58**: 785-791.

Ahmed RF, Abdel-Rahman RF, Farid OaHa, El-Marasy Sa, Hessin AF (2014). Combined hepatoprotective and antidepressant effects of resveratrol in an acute model of depression. *Bulletin of Faculty of Pharmacy, Cairo University* **52**: 191-197.

Andrade SE, Raebel MA, Brown J, Lane K, Livingston J, Boudreau D, Rolnick SJ, Roblin D, Smith DH, Willy ME, Staffa JA, Platt R (2008). Use of antidepressant medications during pregnancy: a multisite study. *American Journal of Obstetrics and Gynecology* **198**: 191-195.

Armstrong WD, Singer L, Makowski EL (1970). Placental transfer of fluoride and calcium. *American Journal of Obstetrics and Gynecology* **107**: 432-434.

Bayne K, Morris TH, France MP (2010). Legislation and oversight of the conduct of research using animals: a global overview. In: R Hubrecht, J Kirkwood, eds., *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*, pp. 107-123. Wiley-Blackwell, Oxford.

Beutler E, Duran O, Kelly BM (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine* **61**: 882-888.

Bilici M, Efe H, Koroglu MA, Uydu H , Bekaroglu M, Deger O (2001). Antioxidative enzyme aztivities and lipid peroxidation in major depression: alterations by antidepressant treatments. *Journal of Affective Disorders* **64**: 43-51.

Fleschler R, Peskin MF (2008). Selective serotonin reuptake inhibitors (SSRIs) in pregnancy: a review. *MCN, The American Journal of Maternal Child Nursing* **33**: 355-361.

Flora SJS, Mittal M, Mishra D (2009). Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic a mines and DNA damage in mouse brain. *Journal of the Neurological Sciences* **285**: 198-205.

Habig WH, Pabst MJ, Jacoby WB (1974). Glutathione-S-transferase, the first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry* **249**: 7130-7139.

Henry ME, Schmidt ME, Hennen J, Villafuerte RA, Butman ML, Tran P, Kerner LT, Cohen B, Renshaw PF (2005). A comparison of brain and serum pharmacokinetics of R-fluoxetine and racemic fluoxetine: a 19-F MRS study. *Neuropsychopharmacology* **30**: 1576-1583.

Karadeniz A, Altintas L (2008). Effects of Panax Ginseng on fluoride-induced haematological pattern changes in mice. *Fluoride* **41**: 67-71.

Maehly AC, Chance B (1954).The assay of catalases and peroxidases. *Methods of Biochemical Analysis* **1**: 357-424.

Mennigen JA, Sassine J, Trudeau VL, Moon TW (2010). Waterborne fluoxetine disrupts feeding and energy metabolism in the goldfish Carassius auratus. *Aquatic Toxicology* **100**: 128-137.

Olivier JD, Vallès A, van Heesch F, Afrasiab-Middelman A, Roelofs JJ, Jonkers M, Peeters EJ, Korte-Bouws GA, Dederen JP, Kiliaan AJ, Martens GJ, Schubert D, Homberg JR (2011). Fluoxetine administration to pregnant rats increases anxiety-related behavior in the offspring. *Psychopharmacology (Berl)* **217**: 419-432.

Park BK, Kitteringham NR, O'Neill PM (2001). Metabolism of fluorine-containing drugs. *Annu Rev Pharmacol Toxicol* **41**: 443-470.

Polakof S, Miguez JM, Moon TW, Soengas JL (2007). Evidence for the presence of a glucosensor in hypothalamus, hindbrain, and Brockmann bodies of rainbow trout. *American Journal of Physiology-Regulatory, Integrative and Comparative* **292**: 1657-1666.

Ramirez AJ, Brain RA, Usenko S, Mottaleb MA, O'Donnell JG, Stahl LL, Wathen JB, Snyder BD, Pitt JL, Perez-Hurtado P, Dobbins LL, Brooks BW, Chambliss CK (2009). Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. *Environmental Toxicology and Chemistry* **28**: 2587-2597.

Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H (1994). Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsome. *Steroids* **59:** 383-388.

Strunecká A, Patoèka J, Connett P (2004). Fluorine in medicine. *Journal of Applied Biomedicine* **2**: 141-150.

Shashi A (2003). Histopathological investigation of fluoride-induced neurotoxicity in rabbits. *Fluoride* **36**: 95-105.

Szasz G (1975). Laboratory measurement of creatine kinase activity. In: N. W. Tietz, A. Weinstock, D. O. Rodgerson, eds., *Proceedings of the Second International Symposium on Clinical Enzymology*, pp. 143-179. American Association for Clinical Chemistry, Washington.

Terri AE, Sesin PG (1958). Determination of potassium in blood serum. *American Society for Clinical Pathology* **29**: 86-89.

Thompson DC1, Perera K, London R (2000) Spontaneous hydrolysis of 4-trifluoromethylphenol to a quinonemethide and subsequent protein alkylation. *Chemico-Biological Interactions* **126**: 1-14.

Vani ML, Reddy KP (2000). Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride* **33**: 17-26.