

## **Influence of Protein Malnutrition on Prenatal Toxicity of Fluoxetine**

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### **Abstract**

**Background:** Protein malnutrition is prevalent in developing countries. Gestational and neonatal malnutrition were considered to contribute to the development of chronic diseases in adulthood.

The aim of the present work was to investigate the influence of prenatal protein malnutrition on embryo -fetal toxic effects of the commonly used antidepressant drug Fluoxetine.

**Methods:** The pregnant rats were divided into two sets: normally fed and protein malnourished. Each set was sub- divided into five groups of 10-14 animals each. Starting from 1st day of pregnancy, animals were fed with standard diet (20% casein). Animals specified for protein malnutrition were switched to protein deficient diet (8%casein) from the 7th day of gestation throughout the end of pregnancy. Fluoxetine hydrochloride was administered orally in the dose levels 2mg and 8mg /kg/day from day 7 to 14 and from day 15 to 20 of gestation. At the 20<sup>th</sup> day of gestation the outcome of pregnancy was examined immediately for viability and fetal growth parameters, placental weight as well as fetal external anomalies. Hb, RBCs count, total and differential WBCs and platelets counts were estimated. Fetuses from each group were subjected to skeletal examinations using the Alcian blue and Alizarin red technique.

**Result:**The results showed that prenatal protein malnutrition and administration of Fluoxetine in the high used dose level 8mg /kg were found to cause significant decrease in fetal growth parameters, and placental weights increase in resorption of fetuses multiple hematomas.

Blood examination of protein malnourished fetuses and groups treated with fluoxetine revealed significant decrease in Hb level; RBCs count & platelets, however, total the leucocytic count was increase. The incidence of skeletal abnormalities was more obvious when fluoxetine was administered during embryogenesis period.

**Key words:** Protein Malnutrition, Prenatal, Fluoxetine

### **Introduction**

Malnutrition occurring during intrauterine period and the first two years of life has adverse effects on child survival and development. When Women in poor areas become pregnant, their nutritional state frequently worsens as the additional demands of pregnancy are not met (De Onis *et al.*, 1998).

Alterations in fetal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology and metabolism of the offspring, thereby predisposing individuals to metabolic, endocrine and cardiovascular diseases in adult life (Barker, 1994).Malnutrition may affect the rate of development by causing premature cessation of cell division and early cell differentiation (Cunningham *et al.*, 1997; Hamed *et al.*, 1998; Harding, 2001; Kramer, 2002 and Villar *et al.*, 2003).

The major components of infant mortality i.e. prenatal and early neonatal mortality are directly

related to the health and nutritional status of the mother during pregnancy. In developing countries, intrauterine growth retardation (IUGR) is an important determinant of Prenatal mortality. Morbidity is also increased in low birth weight newborns.

50% of all low birth weight babies in Egypt can be attributed to IUGR. A negative correlation between neonatal mortality and birth weight was reported (Mansour *et al.*, 1998; LBWSE, 2000).In animals, nutritional deficiency of protein does not cause congenital defects, but can increase prevalence of stillbirth and abortions (Kotera and Madigan, 1997).

Severe degrees of protein deprivation and vitamin deficiencies in mice lead to fetal and placental resorption (Hamed *et al.*,1994).

Prenatal protein malnutrition in rats may delay the change of proteoglycan character which could affect mineralization of fetal bones (Miwa *et al* 1989 & 1990).

Chronic protein malnutrition in humans has a profound detrimental effect on fracture healing (Day and Deheer, 2001).

In a study by Barone *et al.* (1998), they found that low protein diet (4%) in pregnant rat during the second half of

gestation alters copper and iron transport across the placenta and affects copper and iron status of the fetus leading to fetal anemia and hypoproteinemia .

Protein- calorie malnutrition alters drug disposition and its metabolism in human and in animal models (Anderson, 1988). Protein malnutrition was shown to antagonize the behavioral response to chlorpromazine, increases the sensitivity to amphetamine (Hamed *et al.*, 1991) and potentiates the embryo lethal effects of aspirin in rats (Hamed *et al.*, 1994).

This work aims to investigate the influence of prenatal protein malnutrition on embryo-fetal toxic effects of Fluoxetine.

## Material and Methods

Animals 122 Pregnant Sprague Dawley rats were obtained from the animal house of NODCAR. Two females were placed into the cage of one male overnight and presence of sperm in vaginal smears considered the zero day of pregnancy. Mated females were carried and housed in a room maintained at a temperature of  $23\pm 3$  °C and a relative humidity of  $50\pm 10\%$ .

Experimental Design: Standard diet contains 20% casein was given to rats from the first day of gestation (Bamji & Sharada, 1972 and Anthony & Edozien, 1975). The low protein diet (containing 8% casein) was composed of the same constituents of the standard diet except that the amount of casein was reduced to 8g and of sucrose raised to 82g /100g food. Animals of each main group were sub divided into 10 sub groups as follows:

Sub group C1: normal untreated group where animals were fed normal diet (normal fed NF).

Sub group C2: normal untreated group where animals were fed protein malnourished diet

Sub group E1: animals were administered daily 2mg/kg fluoxetine hydrochloride orally from the 7th to the 14th day of gestation and were fed normal protein diet (20% casein).

Sub group E2: animals were administered daily 2mg/kg fluoxetine hydrochloride orally from the 7th to the 14th day of gestation and were fed protein malnourished diet (8% casein).

Sub group E3: animals were administered daily 8mg/kg fluoxetine hydrochloride orally from the 7th to the 14th day of gestation and were fed normal protein diet (20% casein).

Sub group E4: animals were administered daily 8mg/kg fluoxetine hydrochloride orally from the

7th to the 14th day of gestation and were fed protein malnourished diet (8% casein).

Sub group E5: animals were administered daily 2mg/kg fluoxetine hydrochloride orally from the 15th to the 20th day of gestation and were fed normal protein diet (20% casein).

Sub group E6: animals were administered daily 2mg/kg fluoxetine hydrochloride orally from the 15th to the 20th day of gestation and were fed protein malnourished diet (8% casein).

Sub group E7: animals were administered daily 8mg/kg fluoxetine hydrochloride orally from the 15th to the 20th day of gestation while animals were fed normal protein diet (20% casein).

Sub group E8: animals were administered daily 8mg/kg fluoxetine hydrochloride orally from the 15th to the 20th day of gestation while animals were fed protein malnourished diet (8% casein).

At the day 20st of gestation fetuses were removed from the uteri of pregnant rats by cesarean section and number of implantations, resorptions , placental weights (g) and viability of fetuses were recorded.

Fetal growth parameters: body weight (g), crown- rump length (cm), and tail length (cm) were measured. Fetal external examinations and skeletal examinations were done according to the methods of Mcleod, (1980). Hematological parameters of fetuses including hemoglobin level( Hb), erythrocytic count (RBCs), total and differential leucocytic count(TLC) and platelets count were measured.

according to Taylor and Miler method, 1965 England *et al* (1976), Nourbakhsh *et al*, (1978), Hayahoe and Flemans (1982) and Wertz and Koepke (1977) respectively.

Statistical methods: Comparisons between categorical variables were done by the chi-square test (Dawson and Trapp, 2001). All p-values are two sided. P-Values < 0.05 were considered significant.

## Results

No major external abnormalities were detected in the fetuses whether of protein malnourished or normally fed treated rats Effects of fluoxetine and malnutrition on pregnancy

Table 1 showed that the groups of protein malnourished pregnant rats have higher percentage of abortion (36.7%) in comparison to those of normally fed pregnant rats (29%), but the difference between the two groups was statistically insignificant (P-value=0.369). However the highest percentage of abortion (50%) was showed in group administered fluoxetine 8mg/kg daily during the 7th to the 14th days of gestation.

Effects of fluoxetine and malnutrition on the fetuses There are no stillbirths have been

recorded in all groups. However resorbed fetuses were observed in normally fed and protein malnourished groups. The percentage of resorption in normally fed groups was 3.9 % while in protein malnourished groups it was 8.6% (Table 1 and Figs.1&2). The increased rate of resorption among protein malnourished groups compared to normally fed groups was considered statistically significant (P-value = 0.032). However, the highest percentage of resorption (14.3%) was recorded in the group of protein malnourished and treated with fluoxetine 8 mg/kg daily during the 7th to the 14th days of gestation.

External anomalies: Hematomas of variable extent were detected in all treated groups as shown in table 1 and figure 3. Unilateral fore-or hind-limb hematoma was a common observation. Other sites of hematomas were observed at circumsolar region, tip of tail and abdominal wall. Hematomas could be detected in the same fetus in different sites. Severity of hematoma could lead to falling of toes, as noticed in some fetuses of groups (E4) and (E6) in which the percentages of hematomas were 72% and 62.5%, respectively.

Statistical significant difference was observed between normally fed and protein malnourished groups (P-value < 0.001). Fetal growth: Fetal body weights (FBW), fetal crown-rump lengths (FCRL), tail lengths and placental weights are significantly decreased in protein malnourished groups in comparison to normally nourished groups (Table 2 and Fig. 3).

Daily oral administration of fluoxetine, to pregnant normally fed rats in doses 2mg and 8mg/kg during embryogenesis and fetogenesis, produced insignificant differences in FBW, FCR, tail lengths and placental weight compared to normal control. The decrease in FBW observed in control ( $3.34 \pm 0.43$  g) and treated malnourished groups ( $3.32 \pm 0.55$ ,  $3.35 \pm 0.38$ ,  $3.25 \pm 0.34$  and  $3.09 \pm 0.55$ g) was considered statistically significant as compared to normal control ( $3.77 \pm 0.59$ g). Administration of fluoxetine to protein malnourished pregnant rats in a daily dose of 8mg/kg from the 15th to the 20th days of gestation, result in the most affection of mean FBW ( $3.09 \pm 0.55$ g). No statistical significance differences in the fetal body weights were observed in treated protein malnourished rats between drug doses in different gestational periods.

Fetal crown - rump length (FCRL): Table 2 showed that the control group in normally fed rats (C1) had fetuses with mean crown-rump length of  $3.96 \pm 0.23$  cm. Oral administration of fluoxetine daily in 2mg/kg whether in embryogenesis or fetogenesis periods produced insignificant decrease in FCRL as compared to

normal control. While groups E7&F8 (8mg/kg) has significantly decreased FCRL.

In protein malnourished groups, oral administration of fluoxetine daily during the 7th to the 14th days of gestation in doses of 2mg and 8mg/kg produced significant decrease in FCRL as compared to control malnourished group. Oral administration of fluoxetine daily during the 15th to the 20th days of gestation in 2mg/kg produced insignificant decrease in FCRL while significant decrease was observed in 8mg/kg as compared to control malnourished group.

The above observations showed that 8 mg/kg of fluoxetine administered in both gestational periods in N.F and P.M groups caused significant decrement in fetal crown-rump lengths. Dose of 2 mg/kg during the 7th to the 14th days of gestation caused significant decrease in FCRL only in protein malnourished groups.

Fetal tail length (FTL): Oral administration of fluoxetine daily from the 7th to the 14th days of gestation in 2mg/kg produced insignificant decrease in FTL compared to normal control, while the 8mg/kg dose produced significant decrease in FTL. Administration of fluoxetine daily from the 15th to the 20th days of gestation in both doses produced significant decrease in fetal tail lengths compared to normal control (Table 2).

In protein malnourished rats, administration of fluoxetine daily from the 7th to the 14th days of gestation in a dose of 2mg/kg produced insignificant decrease in FTL compared to control malnourished group while a dose of 8mg/kg produced significant decrease. Administration of fluoxetine daily from the 15th to the 20th days of gestation in doses of 2mg and 8mg/kg produced significant decrease in fetal tail length compared to control malnourished group.

Placental weight: Oral administration of fluoxetine daily from the 7th to the 14th days of gestation in 2mg/kg produced insignificant decrease in placental weight compared to control normally fed group, meanwhile the 8mg/kg dose produced significant decrease (Table 2).

Administration of fluoxetine daily from the 15th to the 20th days of gestation in 2mg/kg produced significant decrease in placental weight compared to normally fed control while 8mg/kg induced decrease but statistically insignificant. The control group of protein malnourished rats had mean placental weight of  $0.52 \pm 0.05$  g which was considered significantly lower than control normally fed ( $0.59 \pm 0.07$  g). Administration of fluoxetine daily from the 7th to the 14th days of gestation in the doses of 2mg and 8mg/kg produced insignificant decrease in

placental weight compared to control malnourished. During fetogenesis period (15th-20th day) only the dose of 8mg/kg that produced significant decrease in placental weight.

Hematological parameters: Fetal Hb concentration: Table 3 showed that Hb concentration and RBCs count were significantly lower in fetuses of all groups of protein malnourished (P.M) pregnant rats compared to those of normally fed (N.F). Mean Hb concentration in fetuses of control normally fed mothers (N.F) were 14.9 g%. Mean Hb concentration in the control P.M fetuses were  $11.4 \pm 0.8$  g%.

On administration of fluoxetine in the dose of 2 mg/kg daily during the 7th to the 14th days of gestation in NF and PM rats, the mean fetal Hb concentration was  $14.3 \pm 1.2$  &  $10.8 \pm 1.4$  respectively. Significant decrease in mean fetal Hb concentration occurred on administration of the dose of 8 mg/kg during the same gestational period in NF and PM rats ( $13.6 \pm 1$  &  $9.6 \pm 1.9$  respectively) in comparison to controls.

On administration of fluoxetine in 2 mg/kg daily during the 15th to the 20th days of gestation in N.F and P.M rats, the mean fetal Hb concentration were  $14.6 \pm 1$  &  $10.6 \pm 2$  respectively meanwhile with the 8 mg/kg dose the mean fetal Hb concentration was  $14 \pm 0.8$ . No significant decrease in fetal Hb concentration was observed on administration of fluoxetine in doses of 2 mg/kg during the 15th to the 20th days of gestation in N.F and P.M rats in comparison to controls  $14.6 \pm 1$  &  $10.6 \pm 2$  respectively while with the 8 mg/kg dose the mean fetal Hb conc. was  $14 \pm 0.8$  and  $10.2 \pm 0.8$  respectively.

Fetal RBCs count: Table 3 showed that RBCs count were significantly lower in fetuses of all groups of protein malnourished (PM) pregnant rats compared to those of normally fed (N.F). Mean RBCs counts in fetuses of control normally fed mothers (N.F) was 7.83. Mean RBCs count in the control P.M fetuses was  $5.20 \pm 0.6$ .

On administration of fluoxetine in the dose of 2 mg/kg daily during the 7th to the 14th days of gestation in NF and PM rats, the mean fetal RBCs counts were  $6.43 \pm 1.32$  &  $3.94 \pm 0.47$  respectively. Significant decrease in mean fetal RBCs counts occurred on administration of the dose of 8 mg/kg during the same gestational period in N.F and P.M rats ( $5.73 \pm 1.25$  &  $3.82 \pm 0.34$  respectively) in comparison to controls.

On administration of fluoxetine in 2 mg/kg daily during the 15th to the 20th days of gestation in N.F and P.M rats, the mean fetal Hb conc. & RBCs counts were  $14.6 \pm 1$  &  $10.6 \pm 2$  and  $6.76 \pm 0.56$  &  $4.39 \pm 0.51$  respectively meanwhile with the 8 mg/kg dose

the mean fetal Hb conc. was  $14 \pm 0.8$  and  $10.2 \pm 0.8$  respectively.

There was significant decrease in mean fetal RBCs counts in groups treated with fluoxetine in doses of 2 or 8 mg/kg during the 15th to the 20th days of gestation in N.F and P.M rats in comparison to controls  $6.76 \pm 0.56$  &  $4.39 \pm 0.51$  and  $6.98 \pm 0.46$  &  $3.62 \pm 0.42$  respectively. Fetal platelets count: Table 3 showed that mean fetal platelets count was significantly diminished in protein malnourished groups ( $435 \pm 131$ ) in comparison to normally fed groups ( $654 \pm 173$ ). A significant decrease in mean fetal platelets count was associated with daily administration of fluoxetine in 8mg/kg during the 15th to the 20th days of gestation in both normally fed ( $477 \pm 110$ ) and protein malnourished groups ( $388 \pm 115$ ).

Fetal total and differential leucocytic Counts (TLC):

Table 3 showed that mean fetal total leucocytic count (TLC) was higher in protein malnourished groups ( $12.4 \pm 4.4$ ) in comparison to normally fed groups ( $10.2 \pm 2.7$ ).

The mean fetal TLC was very high in protein malnourished groups treated with fluoxetine in comparison to normally fed groups except in a dose of 8 mg/kg daily at the 15th to the 20th days of gestation.

The increase of total leucocytic counts was associated with an increase of lymphocytes% and proportional decrease of neutrophils%.

Fetal skeletal system: Fetal skeletal abnormalities were most obvious in fetuses maternally treated during the 7th -14th day of gestation as shown in Table 4 and figures 4&5.

The skeletal defects observed in fetuses included lack of ossification of skull bones, sternum, ribs, vertebrae, fore limbs bones, pelvic and hind limbs bones (Fig. 5B).

The major skeletal defects were observed mainly in pelvic and hind limbs bones in the form of shortness and partial ossification of ilium ischium, pubis, femur, tibia and fibula (Fig. 4B). Tortuosity and shortness of the 13th rib were observed in some fetuses (Figs. 4B & 5A & B). Incomplete ossifications of vertebral column were observed mainly in sacral and caudal vertebrae (Fig 5B). Forelimbs bones were less affected than hind limbs bones. Abnormalities detected in forelimbs bones were in the form of partial ossifications of humerus, ulna, and radius and missed ossifications of metacarpals and phalanges (Fig. 5).

in almost groups was observed in (Fig. 5). The most affected group was the group of

fetuses of protein malnourished pregnant rats that are administered fluoxetine in a dose of 8mg/kg during the 7th to the 14th days of gestation. The bone defects observed were

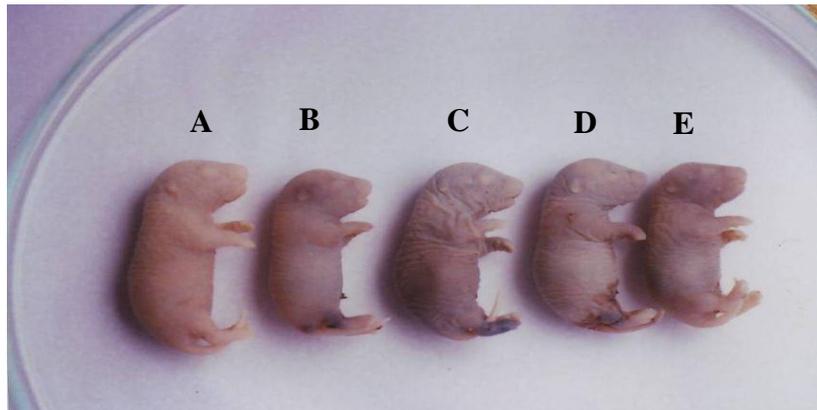
mainly partial ossifications of hind limbs bones in 90% of fetuses, which are extended to the forelimbs in 50% of them (Fig 4B). The least effects on bones were observed in fetuses maternallyexposed to fluoxetine (8mg/kg)



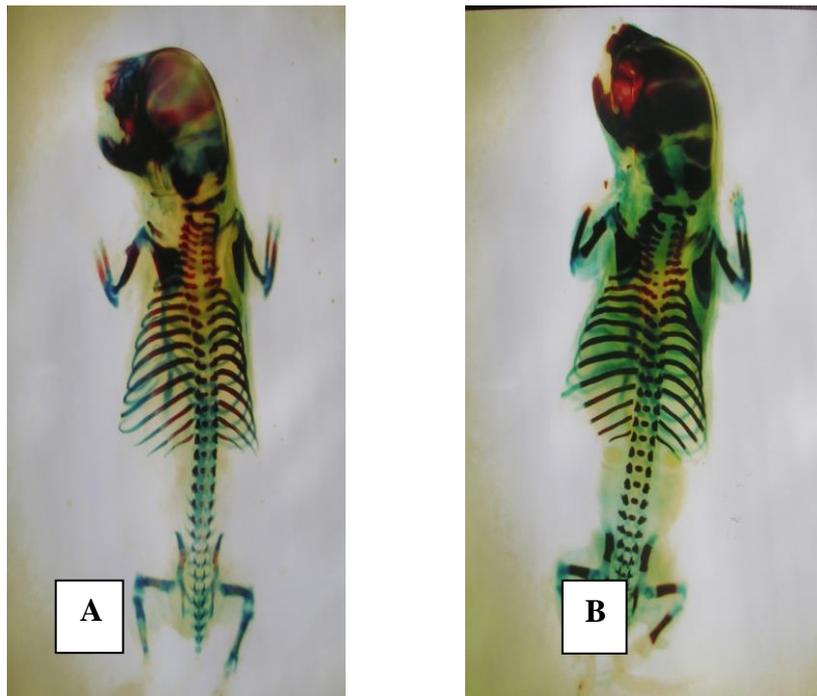
**Fig (1):** A photograph of two uteri of pregnant rats at the 21<sup>st</sup> day of gestation showing:  
A) Normal symmetrical uterine horns of a control N.F pregnant rat.  
B) Asymmetrical uterine horns of a N.F pregnant rat treated with 8mg/kg of fluoxetine at (7-14 days) of gestation.



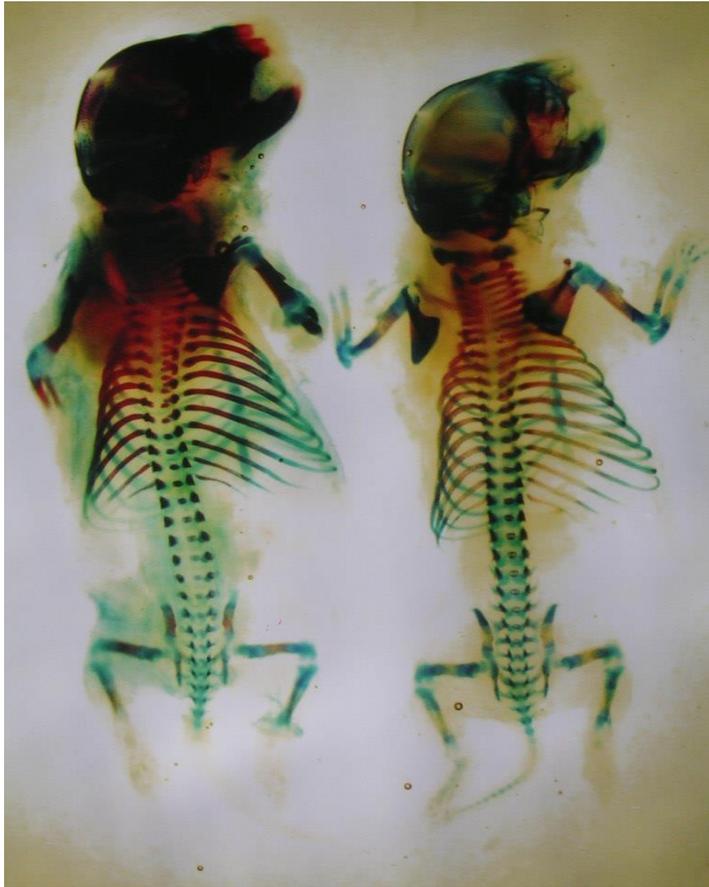
**Fig (2):** A photograph of uteri of two P.M. pregnant rats at the 21<sup>st</sup> day of gestation treated with 8mg/kg of Fluoxetine at (7-14 days) of gestation showing:  
A) Hemorrhagic spots denoting abortion. B) Complete resorption of fetuses.



**Fig (3):** A photograph of fetuses at the 20<sup>th</sup> day of gestation showing that (A) control malnourished fetus, (B, C & D, E) protein malnourished fluoxetine treated fetuses by 2 and 8 mg/kg during embryogenesis and fetogenesis, respectively, showing multiple hemorrhagic sites with variable degrees of severity.



**Fig(4):** Two photographs of skeletons of two full term NF fetuses: control(A) and treated by 8mg/kg of FX from 7-14d of gestation (B) showing defective ossification in skull bones, sacral, pelvic, hind limb bones as well as metacarpals and metatarsals in comparison to control.



**Fig (5):** A photograph of dorsal aspects of two skeletons of full term PM fetuses treated by 8/mg of FX from 7-14 d of gestation showing that the fetuses have missed ossification of phalanges fore and hind limbs and lack of ossification in pelvic girdles and ribs.

**Table (1):** Effects of oral administration of fluoxetine on the outcome of pregnancy and fetuses in normally fed and protein malnourished groups

Groups	No of pregnant rats	Abortion		No of mothers	Total implant.		No of alive fetus	Resorption		External anomalies (hematoma)	
		No	%		No	Aver. /mother		No	%	No	%
NF Control (C1)	10	1	10	9	62	6.9	60	2	3.2	-	-
FX(2mg7-14 d)(E1)	10	3	30	7	47	6.7	45	2	4.3	21	14.7
FX(2mg15-20 d)(E2)	14	2	14.2	12	54	4.5	52	2	3.7	22	42.3
FX(8mg7-14 d)(E3)	14	6	42.9	8	44	5.5	42	2	4.6	14	33.3
FX(8mg15-20 d)(E4)	14	6	42.9	8	52	6.5	52	2	3.8	36	72
PM Control (C2)	10	2	20	8	39	4.8	37	2	5.4	7	18.9
FX(2mg7-14 d)(E5)	12	4	33.3	8	48	6	45	3	6.3	18	40
FX(2mg15-20 d)(E6)	12	4	33.3	8	52	6.5	48	4	7.7	30	62.5
FX(8mg7-14 d)(E7)	14	7	50	7	35	5	30	5	14.3	13	43.3
FX(8mg15-20 d)(E8)	12	5	41.6	7	36	5.1	32	4	11.1	7	21.9

**Table (2):** Changes in fetal body weights; fetal crown –length and tail length induced by fluoxetine in normally fed (NF) and protein malnourished (PM) rats.

Drug	fetal body weights (g)		fetal crown – length (cm)		fetal tail length (cm)		placental weight (g)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Control N. F. (C1)	3.77	0.59	3.96	0.23	1.27	0.11	0.59	0.07
Flx. 2mg/kg (at 7-14 days) (E1)	3.58	0.40	3.82	0.30	1.23	0.09	0.56	0.09
Flx. 2mg/kg (at 15-20 days) (E2)	3.58	0.23	3.94	0.27	1.21	0.07	0.49	0.05
Flx. 8mg/kg (at 7-14 days) (E3)	3.70	0.58	3.76	0.16	1.20	0.08	0.48	0.13
Flx. 8mg/kg (at 15-20 days) (E4)	3.73	0.66	3.69	0.21	1.18	0.05	0.54	0.04
Control P. M. (C2)	3.34	0.43	**2.39	0.11	1.27	0.07	0.52	0.05
Flx. 2mg/kg (at 7-14 days) (E5)	3.32	0.55	**2.34	0.09	1.23	0.08	0.46	0.11
Flx. 2mg/kg (at 15-20 days) (E6)	3.35	0.38	**2.26	0.07	1.21	0.07	0.47	0.10
Flx. 8mg/kg (at 7-14 days) (E7)	*3.25	0.34	**2.32	0.08	1.20	0.10	0.49	0.10
Flx. 8mg/kg (at 15-20 days) (E8)	*3.09	0.55	**2.26	0.05	1.18	0.07	0.43	0.10

**Table (3): Effects of protein malnutrition and fluoxetine administration on hematological parameters of fetuses.**

Drug	Hemoglobin in conc.g/100	RBCs (million/cmm)	platelets count (1000/cm <sup>3</sup> )	Total leucocytic count(1000/cmm)	Lymphocytic Count/100 cells	Neutrophilic Count/100 cells
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)
Control N. F. (C1)	14.9 (1.8)	7.83 (1.43)	654 (173)	10.2 (2.7)	72.0 (6.1)	11.6 (3.4)
Flx. 2mg/kg (at 7-14 days) (E1)	14.3 (1.2)	6.43 (1.32)	675 (124)	8.8 (1.3)	72.5 (5.6)	13.0 (2.9)
Flx. 2mg/kg (at 15-20 days) (E2)	14.6 (1.0)	6.76 (0.56)	622 (111)	9.4 (1.3)	71.8 (4.2)	12.4 (3.0)
Flx. 8mg/kg (at 7-14 days) (E3)	13. (1.0)	5.73 (1.25)	535 (139)	11.5 (3.6)	86.6 (7.5)	9.0 (5.4)
Flx. 8mg/kg (at 15-20 days) (E4)	14.0 (0.8)	6.98 (0.46)	477 (110)	18.5 (4.2)	80.7 (18.6)	7.2 (3.5)
Control P. M. (C2)	11.4 (0.8)	5.20 (0.67)	435 (131)	12.4 (4.4)	81.3 (7.2)	9.2 (4.1)
Flx. 2mg/kg (at 7-14 days) (E5)	10.8 (1.4)	3.94 (0.47)	457 (124)	14.9 (3.0)	81.1 (4.7)	11.6 (3.4)
Flx. 2mg/kg (at 15-20 days) (E6)	10.6 (2.0)	4.39 (0.51)	408 (133)	16.2 (5.3)	78.4 (8.5)	12.4 (3.8)
Flx. 8mg/kg (at 7-14 days) (E7)	9.6 (1.9)	3.82 (0.34)	406 (113)	20.7 (4.9)	88.5 (3.2)	6.9 (2.4)
Flx. 8mg/kg (at 15-20 days) (E8)	10.2 (0.8)	3.62 (0.42)	388 (115)	9.0 (1.4)	74.3 (4.1)	16.2 (5.3)

\*significant P&lt; 0.01

\*\*highly significant P&lt; 0.001

**Table (4): Incidence of fetal skeletal malformations induced by fluoxetine in normally fed and protein malnourished rats**

Groups	No. of alive fetuses	Abnormalities%											
		Inter-parietal bone of the skull (P.O)	5 <sup>th</sup> and 6 <sup>th</sup> sternbrae (P.O)	Forelimbs bones				Hind limbs bones					
				Humerus (P.O)	Radius and Ulna	Metacarpals	Phalanges (M.O)	Ilium	Pubis and	femur (P.O)	Tibia and	Metatarsals (M.O)	Phalanges (M.O)
NF Control (C1)	60	-	-	-	-	-	-	-	-	-	-	-	-
FX(2mg7-14 d)(E1)	45	70	70	30	30	30	90	50	50	60	60	70	60
FX(2mg15-20 d)(E2)	52	50	50	10	10	10	90	30	30	50	50	40	60
FX(8mg7-14 d)(E3)	42	60	80	-	-	-	60	60	60	80	80	60	10
FX(8mg15-20 d)(E4)	52	30	30	-	-	20	90	40	40	70	70	80	10
PM Control (C2)	37	10	30	-	10	-	80	-	-	10	10	20	50
FX(2mg7-14 d)(E5)	45	-	-	-	-	-	60	-	-	-	-	-	20
FX(2mg15-20 d)(E6)	48	-	-	-	-	-	70	10	10	10	10	-	40
FX(8mg7-14 d)(E7)	30	80	80	50	50	80	10	90	90	90	90	100	100
FX(8mg15-20 d)(E8)	32	-	-	-	-	-	80	-	-	-	-	10	70

M.O: denotes miss ossified  
percentage (%)

P.O: denotes partially ossified

The data represented as

## Discussion

Since the pioneering work of Eward *et al.* (1914) demonstrating that maternal dietary protein deficiency resulted in lower birth weights and decreased vigor of the offspring in pigs, there have been extensive studies of the effects of dietary protein restriction on fetal growth in humans, pigs and rats

(Pond *et al.*, 1992; Schoknecht *et al.*, 1994; Jain *et al.*, 1995; Desai *et al.*, 1996). Collectively, these studies have shown that protein deficiency during early or mid-gestation results in decreased placental and fetal growth and may permanently retard postnatal growth.

Amino acids are not only the building blocks of proteins and peptides, but also essential precursors for the synthesis of important molecules such as hormones, neurotransmitters, purine and pyrimidine nucleotides, polyamines, creatine, carnitine, nitric oxide (NO) (Moncada and Higgs, 1993) and porphyrins (Reeds and Hutchens, 1994). NO synthesized from arginine by NO synthase, was reported to be essential for fertilization (Herrero *et al.* 1996), embryo attachment and development in the uterus (Norman 1996, Novaro *et al.* 1997) and plays a critical role in regulating uterine blood flow and thus nutrient supply to the fetus during gestation (Sladek *et al.* 1997).

In this study the percentage of resorptions in normally fed control group was 3.2% while in the protein malnourished one it was 5.4%.

The mean body weight of control normally fed group was  $3.77 \pm 0.5$  g while in protein malnourished control group it was  $3.34 \pm 0.43$ g. Mean fetal crown-rump length and fetal tail length were significantly decreased in control malnourished group compared to normally feed one.

Mean placental weight was significantly reduced in control malnourished group. Levy and Jackson (1993) reported a decrease in the number of viable fetuses and an increase in the number of resorptions in pregnant rats feeding 6% and 9% protein diet. The placental weight was not different to the 18% group and on the 12% diet, but was significantly increased on the 9% diet and significantly decreased on the 6% diet. Fetal weight was greatest on the 12% diet and significantly decreased on the 9% diet. The decrease in concentration of essential and non-essential amino acids in the fetus may be a mechanism whereby maternal protein restriction results in fetal growth retardation (Guayao *et al.* 1998). In the present study, fetuses of control malnourished group have missed ossification of phalanges of fore and hind limbs in comparison to normal control. Some of these fetuses showed defective skull

and vertebral cartilage formation. According to Miwa *et al.* (1990) prenatal protein malnutrition may delay the changes of proteoglycan character, which could affect the mineralization of fetal bones.

In this study Hb level and RBCs count were significantly lower in protein malnourished control group in comparison to fetuses of control normal group. Total leucocytic count was higher in fetuses of malnourished control group in comparison to fetuses of normally nourished control group. The increase of total leucocytic count was associated with an increase in the lymphocytes % and proportional decrease in the neutrophils %. Maternal protein malnutrition is a regulatory factor in fetal mineral homeostasis. Maternal low protein diet may affect transport of iron across the placenta leading to anaemia (Barone *et al.*, 1998). Amino acids are essential precursors for the synthesis of porphyrins (Reeds and Hutchens, 1994). Decrease in amino acid content of maternal diet may lead to decrease in synthesis of hemoglobin (Cosens *et al.*, 1977). Increased total leucocytic count and lymphocytes % may be due to immaturity of the fetus as a result of maternal malnutrition (Davies *et al.*, 1992).

The present work showed that administration of fluoxetine (FX) to normally fed pregnant rats during the period of organogenesis (7 – 14 days) and the last week of gestational period (15 – 20 days) resulted in increased incidence of abortion compared to normal control pregnant rats. This increase was dose-dependent. This agrees with Koren *et al.*, (2005) who demonstrated that antidepressants used by pregnant women may be associated with increased risk for spontaneous abortion. Also, Fluoxetine and its metabolites were found to traverse the placenta and distribute within the conceptus during periods of organogenesis and post organogenesis in Wistar rats (Pohland *et al.*, 1989).

In the present study, increased percentage of resorbed fetuses was observed specially in normally fed rats treated with 8mg/kg accordance with Carey and McLaughlin fluoxetine during organogenesis period. This was in (2002), who observed prevalence of stillborn pups and pups with reduced birth weight and decreased viability in rats exposed to  $\geq 1.5$  times the maximum recommended dose of fluoxetine in human.

In the present study, although fetal body weight was not significantly affected by time of administration or by doubling the dose of fluoxetine, one must be careful to evaluate the data in light of other known factor influencing this parameter. For instance, fetal weight is

inversely proportional to litter size in mice, rats and rabbits (**Hafez, 1963; McLaren, 1965**).

In our study Fluoxetine resulted in significant reduction of FCRL and tail lengths when administered to normally fed rats, in a dose of 8 mg / kg during late gestational period.

Different doses of fluoxetine (2, 5, 12.5mg / kg) were given orally to pregnant rats by **Byrd and Markham (1994)**. Maternal toxicity was observed only, at dose of 12.5 mg /kg by reduction of weight gain and food consumption. The authors added that fetal viability, weight and morphology were not affected by smaller doses. This was confirmed by **Vorhees *et al.*, (1994)**, who reported that giving a dose of 12.5 mg / kg fluoxetine resulted in obvious loss of maternal weight, reduced litter sizes at birth and increased neonatal mortality.

**Chambers *et al.* (1996)** demonstrated an increased risk of premature birth and lower birth weight when fluoxetine was given to pregnant women during the last trimester compared to the first trimester.

Fluoxetine (FX) is a selective serotonin reuptake inhibitor (SSRI) which increases serotonin neurotransmission. Serotonin is involved in the regulation of a variety of physiological systems, including the sleep – wake cycle, circadian rhythms and the hypothalamic–pituitary–adrenal axis. Each of these systems plays also an important role in fetal development. FX causes an acute increase in plasma serotonin level, leading to a transient reduction in uterine blood flow. This, in turn, reduces the delivery of oxygen and nutrients to the fetus, thereby presenting a mechanism for reducing growth and / or eliciting preterm delivery. Moreover, because FX crosses the placenta, the fetus is exposed directly to FX as well as to the effects of the drug on the mother. (**Morrison *et al.* 2005**).

In the present work no major or minor fetal malformations were detected by external and internal examination of normally nourished fetuses exposed to different doses of fluoxetine in both gestational periods. These results were in agreement with the available animal and human experience with fluoxetine, which appeared to indicate that the antidepressant was not related to major congenital malformation (**Pastuszak *et al.* 1993; Addis and Koren 2000**).

In the present study dose-dependent hematomas were observed in normally fed (N.F) treated fetuses in comparison to control. In a study by **Stanford and Patton (1992)**, higher frequency of skin hematomas were observed in rat fetuses subjected to fluoxetine (5.6 mg/kg/day) from day 7<sup>th</sup> of gestation until delivery. The mechanism was thought to be related to the

inhibition of serotonin uptake by platelets or vascular instability.

In the present study, skeletal examination of N.F fetuses maternally exposed to 2mg and 8mg/kg of fluoxetine during both gestational periods revealed incomplete ossification of bones of skull, pelvis and hind limbs, moreover absence of small bones of fore and hind limbs. Daily administration of fluoxetine from 7<sup>th</sup> to 14<sup>th</sup> days of gestation had more influence on bone formation than late gestational period.

**Shuey *et al.*, (1992)** mentioned that inhibition of 5-HT uptake into craniofacial epithelia of mouse embryo at 9-12 days of gestation might produce developmental defects by interference with serotonergic regulation of epithelial-mesenchymal interactions important for normal cranio-facial morphogenesis. Skeletal changes induced by fluoxetine in the present work were in accordance with **Warden *et al.*, (2005)** who studied the effects of fluoxetine on the growing skeleton in mice and found a reduction in bone formation without an increase in bone resorption and these effects were not influenced by serum biochemistries. They explained that fluoxetine might inhibit serotonin transporter, which was present on osteoclasts and played a critical role in the differentiation of these cells. **Mundell (2004)** reported that fluoxetine may stunt growing bone of mice.

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## اثر نقص البروتين على سمية عقار الفلوكستين عند استعماله في فترة الحمل على المواليد

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يمثل سوء التغذية البروتيني مشكلة اجتماعية في أنحاء متفرقة من العالم وعلى وجه الخصوص الدول النامية.

تهدف هذه الدراسة إلى معرفة مدى تأثير نقص البروتين في الغذاء على قدرة عقار فلوكستين وهو من الأدوية المضادة للاكتئاب لإحداث تأثيرات سمية واحتمالات إحداث تشوهات في أجنة الجرذان.

وقد استخدم في هذا البحث 122 من الجرذان الحوامل تم تقسيمها إلى مجموعتين رئيسيتين: عادية التغذية والأخرى ناقصة البروتين في الغذاء وكل منهما قسم إلى خمس مجموعات فرعية.

اعتباراً من اليوم الأول من الحمل تم تغذية الجرذان على غذاء عياري يحتوى على 20% كازيين ومن اليوم السابع للحمل بدأت تغذية المجموعة الثانية (ناقصة البروتين في الغذاء) بغذاء يحتوى على 8% كازيين حتى نهاية الحمل.

تم معالجة الجرذان الحوامل بواسطة الفم بجرعتين مختلفتين وهي 2ملجم، 8ملجم/ كجم من الفلوكستين يومياً على مرحلتين الأولى من اليوم السابع حتى اليوم الرابع عشر والثانية من اليوم الخامس عشر حتى اليوم العشرين من الحمل.

تم ذبح الإناث الحوامل في اليوم الواحد والعشرين، وفي الحال تم إختبار الآتي: وزن المشيمة والأجنة الحية وكذلك ملاحظة التشوهات الخارجية للأجنة.

تم فحص الدم لبعض الأجنة من كل مجموعة من المجموعات العشرة وذلك بقياس نسبة الهيموجلوبين وعدد كرات الدم الحمراء والبيضاء والصفائح الدموية.

عشرة أجنة من كل مجموعة من المجموعات العشر استخدمت لفحص الهيكل العظمي والغضروفي باتباع طريقة صبغة الألبان الزرقاء و الاليزارين الحمراء

النتائج التي تم الحصول عليها من خلال الدراسة كانت كالاتي :

- نقص البروتين قلل من وزن المشيمة ووزن الأجنة وطول الجسم وكذلك طول الذيل في الاجنة. إعطاء الفلوكستين بالجرعة العالية 8 ملجم/ كجم أدى إلى نقص واضح في طول
- الأجنة وطول الذيل ووزن المشيمة في فترات الحمل المختلفة وهذا النقص أكثر وضوحاً في مجموعات الجرذان ناقصة البروتين في الغذاء.
- نقص البروتين في الغذاء أدى الي زيادة عدد الأجنة الممتصة في المجموعات ناقصة التغذية البروتينية.
- الفحص الظاهري للأجنة في كل المجموعات سواء مجموعات التغذية العادية أو مجموعات التغذية ناقصة البروتين أظهر بعض البقع الدموية في الأطراف بنسب مختلفة كما أظهر عدم وجود تشوهات ظاهرية.
- نقص البروتين في غذاء الامهات الحوامل أدى الي خفض نسبة الهيموجلوبين وعدد كرات الدم الحمراء و زيادة عدد كرات الدم البيضاء (وبالأخص ليمفوسيت) في اجنة مجموعات الامهات ناقصة التغذية البروتينية بالمقارنة بالمجموعات عيارية التغذية. عدد صفائح الدم قد نقص في اجنة

مجموعات الامهات ناقصة التغذية البروتينية بالمقارنة بالمجموعات عيارية التغذية (0 إعطاء الفلوكستين بالجرعة العالية 8ملجم/ كجم من اليوم السابع حتى اليوم الرابع عشر للحمل أدى إلى زيادة النقص في الهيموجلوبين وعدد كرات الدم الحمراء و زيادة عدد كرات الدم البيضاء فى اجنة مجموعات الامهات ناقصة التغذية البروتينية والمجموعات عيارية التغذية.

- فحص التراكيب الهيكلية للأجنة أظهر زيادة كبيرة فى تشوهات العظام فى المجموعات ناقصة التغذية البروتينية وعند اعطاء الفلوكستين من اليوم السابع حتى اليوم الرابع عشر من الحمل وقد كانت التشوهات عبارة عن نقص التعظم فى تكوين عظام الجمجمة و الحزام الحوضي والفخذ وعظام الساق وعظام هيكل القدم واليد.