The Egyptian Journal of Hospital Medicine Vol., 21: 16 – 32 December 2005
I.S.S.N: 12084
2002–1687

Toxic effects of paroxetine on sexual and reproductive functions of rats

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Abstract

This study determined some appropriate parameters to detect male and female reproductive, endocrine, and teratological toxicity besides genotoxic effects of paroxetine. Several parameters concerning fertility were measured in male and female rats given oral doses of paroxetine (0.36mg/100g b.wt./day) for a month. In addition, estimation of the drug residues in male, female and fetus were carried out. There were alterations in serum concentration of gonadotraphins: FSH, LH, PRL and in the sex hormones : testosterone, progesterone and estradiol in male and female rats. These alterations were accompanied by sperm reduction, sperm abnormalities, irregular shaped of seminiferous tubules, hypertrophy of geominal cells and structural chromosomal aberrations in male rats. A reduction in mating and fertility indices were observed with an increase in the number of resorbed fetuses, and a decrease in body weight and crown rump length of rat embryos.

Introduction

Paroxetine is one of the mostly used antidepressant drugs in recent years. It is a short acting selective serotonin reuptake inhibitor (SSRI) Perry (2004). As the name implies, the primary pharmacologic effect of the SSRIs is to block the presynaptic serotonin transporter receptor . SSRIs are more potent inhibitors of serotonin reuptake, and they have less effect on a_1, a_2, histaminic, and muscarinic receptors. Paroxetine blocks the uptake of serotonin into human platelets (Grimsley *et al.*, 1992). Studies in animals also showed that the drug is a potent and highly selective inhibitor of neuronal serotonin reuptake as it has only very weak effects on norepinephrine and dopamine neuronal reuptake (Swaby,1995). Paroxetine has no active metabolites, and a half-life of approximately one day. It is classified as a Pregnancy Category B drug (Swaby, 1995). SSRI-induced changes in sexual function that can actually be beneficial in some clinical situations (Lee *et al.*, 1996).

Sexual dysfunction is a common side effect of SSRIs, occurring in more than 30% of patients. Sexual side effects have emerged as a major clinical concern with many of the newer antidepressants. Approximately 30% to 40% of patients on serotonergic antidepressants experience sexual dysfunction (Michael, 1998). From the manufacturers data estimates of the incidence of sexual dysfunction resulting from SSRIs ranges from 1.9-15.9% (Perry, 2004).

There is also sufficient evidence that all psychotropic drugs readily cross the placenta to reach the fetus and may be excreted into breast milk (Chisholm and Kuller, 1997; Arnon *et al*., 2000; Bar-Oz *et al*., 2000). Drugs in the fetus may have a higher unbound free fraction, easily penetrate into the brain, and undergo only limited hepatic and/or extrahepatic metabolism (Arnon *et al*., 2000; Hines and McCarver, 2002; McCarver and Hines, 2002). Fetus, or infant exposure to

psychotherapeutic drugs against the risks, for both mother and offspring, of untreated mental disorders is a real point of dispute (Altshuler *et al.,* 1996; Kuller *et al.,* 1996; Koren *et al.,* 1998).

Aim of the work

The aim of the present work was to evaluate the genotoxicity of a clinical dose of paroxetine (0.36mg/100g b.wt.) on male fertility and on pregnant female rats and their embryos. Chromosomal aberrations in the bone marrow cells of males, changes in sperm analysis , histopathology of testis , endocrine function and the level of the drug in the fetus and mother were also determined.

Materials and Methods

Drug:

Paxil (paroxetine hydrochloride , tablets) produced by Bristol Myers Squibb Company, Cairo , Egypt was used in this study. It is the hydrochloride salt of a phenylpiperidine compound identified chemically as (-)-trans-4R-(4'-fluorophenyl) -3S-[(3',4'- methylene-dioxyphenoxy) methyl] piperidine hydrochloride hemih-ydrate and has the empirical formula of C19H20FNO3·HCl·1/2H2O. The molecular weight is 374.8 (329.4 as free base). Paroxetine hydrochloride is an odorless, off-white powder, having a melting point range of 120° to 138°C and a solubility of 5.4 mg/ml in water.

Animals:

Healthy mature male and female albino rats (Rattus norvigicus) were obtained from Abu- Rowash were used. They were of 150-170g body weight. The animals were fed on a well-balanced ration and water ad-libitum and kept under constant environmental conditions. Male rats were divided into four groups. Three groups were given oral doses of paroxetine (0.36 mg/100 g b. wt./day) for 10, 20 and 30 days and the fourth served as control. These daily dose was equal to the therap-eutic dose (40 mg) in man . The doses were calculated for rats according to Paget and Barnes (1964) species interconversion of dosage .

Female rats were divided into 2 groups, one of them administered oral doses of paroxetine (0.36 mg/100 g b. wt./day) for 10 days and the other served as control. Treated and untreated females in estrus phase were paired over night with males. At the next morning, vaginal smears were examined microscopically. Insemination was confirmed by detecting the sperm-atozoa in the vaginal smears. Females with positive vaginal smears (presence of spermatozoa) were considered pregnant and the day of detection was defined as "Day 0" of pregnancy.

Mating tests were divided into:

1-Unreated male and untreated female.
2- Untreated male and treated female.
3- Treated male and untreated female.
4- Treated male and treated female.

Pregnant females were administered oral doses of paroxetine (0.36 mg/100g b. wt./ day) from 5-19 day of the gestation days. A group of pregnant females was kept as control. All groups except that of colchicine were sacrificed two hours after drug administration at the time points. Blood samples were collected, sera were separated and internal organs were removed for histopathological examination.

Biochemical Analysis:

Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), testosterone, progesterone and estr-adiol (E_2) concentrations were determined using enzyme linked immunosorbant assay (ELIZA) kits, BioCheck, Inc. from Medicopharmatrade, Egypt.

Teratological study:

Females were sacrificed on day 19 of gestation and fetuses were counted weighed, and the number of alive, resorbed, and dead fetuses were recorded. Fetuses were examined externally and internally to investigate any abnormalities. Also, fetuses were stained in Alizarin red – S according to Weesner (1986) and skeletons of fetuses were examined.

Cytogenetic study:

After the treatment schedule of paroxetine, male rats were injected intraperitoneally with colchicine (4mg/kg b.wt.) and sacrificed two hours later. The femur bones were quickly separated. Chromosomes of bone marrow cells were prepared according to a modified method of Luck and Tice (1987). Chromosomal aberration assay was performed by screening fifty well metaphases per animal for scoring different types of aberrations (Hoffmann,1996). The metaphases were scored according to Venitt and Parry (1984) for chromosome- type aberration, gaps, breaks, fragments, exchanges, dicentric, rings. The significance of differences between the experimental and control data was calculated using Student's t-test.

Sperm Analysis

The caudal epididymis was dissected, semen collected and subsequently diluted with saline solution and placed in Burker Turk haematocytometer. Sperm production in the testis was assessed by sperm head counting based on the procedure of Meistrich (1989). The sperms were examined morphologically and the results were evaluated statistically.

Histopathological Examination:

Treated and control male rats were sacrifcied and dissected on 10, 20, and 30 days and right testes were picked out.Testes were fixed in 10% neutral Buffer formalin. Paraffin sections of 4 – 6 micrometer thickness were prepared and stained by Hematoxylin and Eosin for histological examination (Drury and Wellington, 1980).

Toxicological Analysis:

Estimation of the residual paroxetine in liver and kidney of the treated rats(males and females) and the whole fetuses were carried out.

(A) Extraction Method:

For extraction of the drug, ammonium sulfate method was used according to Nickolls (1956). A tissue sample was accurately weighed, homogenized with glass powder and diluted with half its weight water, and then equal volume of 10% hydrochloric acid was added. An excess of ammonium sulfate crystals was added to saturate the homogenate with continuous stirring and heating over a boiling water bath. The mixture was left over night to ensure complete penetration of the extracting fluid into the cells of the homogenized tissue. Ethyl acetate solvent was used for extraction. The extract was dehydrated over sodium sulfate then filtered and evaporated to dryness in vacuo at 60 ºC. For quantitative analysis the residue was dissolved in methanol. The apparatus used was a Model Hewlett Packard 5089 (H P 5089) gas chromatography with a FID (flame ionization detector). The column was HP 5 capillary column 28 meter(m) in length with a diameter 0.32 µm. the carrier gas was nitrogen. The flow rate was 3 ml/minute. Detector temperature was 325°C, injector temperature was 315°C, and oven temperature was isothermal 280°C. Retention time was 2.46 minutes.

(B)Quantitative analysis:

Five different drug concentrations were prepared .The peak area of each concentration was calculated, and a calibration curve was plotted using peak area against concentration. By using the calibration curve for the studied drug, the concentrations of the drug in residues of different tissues were obtained.

Statistical analysis:

Statistical analysis was performed using Student's t- test and all the results were expressed as means$\pm$ standard error. $P<0.05$ was considered significant.

Results and Discussion:

Data presented in Table (1) revealed that paroxetine (0.36 mg /100g b.wt. orally) significantly reduced serum LH, FSH, testosterone, but elevated prolactin and estradiol in male rats after 20 and 30 days of treatment. 10 days treatment showed a slight reduction in testosterone and a slight elevation in prolactin levels.

Data presented in Table(2) indicated that paroxetine administration for 10 days in female rats reduced significantly serum concentration of LH, FSH, progesterone

and estradiol while increased prolactin and testosterone compared with untreated females (controls). At the same time pregnant females administered paroxetine from 5-19 days of gestation showed reduced levels of progesterone and estradiol compared with control pregnant females.

The activity of the gonads (testes or ovaries) is regulated by the pituitary gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Secretion of both hormones is controlled by gonadotrophin-releasing hormone (GnRH) from the hypothalamus. Primary drug-induced infertility results from a direct toxic effect of the drug on the gonads or an indirect effect on the pituitary gland (Maclean and Lee,1999).

There are many reports that antidepressant medications often interfere with several parts of sexual response in males and females (Maclean and Lee, 1999; Ferguson, 2001; Smith, 2004). SSRIs antidepressants increase serotonergic neurotransmission and serotonin plays a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, which can subsequently lead to altered secretion of ganadotrophin and releasing factors thus affecting reproductive behaviour (Hendrick *et al.*, 2000; Marrison *et al*., 2004). There is evidence of dopaminergic, adrenergic, muscarinic and serotonergic involvement. In general, dopamine increases sexual behaviour and serotonin inhibits it (Maclean and Lee, 1999).

Rhoades and Tanner (2003) reported that in males hypogonadotrophin hypogonadism, the primary abnormality is in the hypothalamus or the pituitary. There may be selective deficiency of gonadotrophin production, of both LH and FSH, or only LH. Alternatively, the deficiency may be part of a generalized failure of pituitary hormone production. Plasma LH and testosterone are both reduced, and usually there is low plasma FSH as well. This is in accordance with the present results which indicated the reduction of FSH, LH and testosterone in male rats at the 20^{th} and 30^{th} days of paroxetine treatment. The present data are also supported by the work of Daoud *et al.* (2004) who reported a significant reduction in both testosterone and FSH levels in antiepleptics- fed male rats.

The elevation of prolactin commonly occurs with taking antidepressant drugs (Jacobs, 2002). Broadbear *et al*. (2004) also reported prolactin increase after sertraline administration in male and female sheep. These findings supported the present results.

Although estradiol constitutes only 1% of the total gonadal steroids in men, it exerts almost 50% of the negative feedback on the hypothalamus. Therefore, even small elevation in estradiol levels may have relatively profound suppressive effects on testosterone synthesis and sexual function (Herzog *et al*., 2004). This is in accordance with the present data in which paroxetine administration elevated estradiol levels and reduced testosterone.

In the present study administration of 0.36 mg paroxetine/100g b.wt. for 10 days in female rats significantly reduced serum FSH, LH, progesterone and estradiol with an elevation of prolactin and testosterone.

As mentioned above for males, the female hormonal system is also affected by SSRIs (Maclean and Lee, 1999; Ferguson, 2001; Smith, 2004). Hypothalamic and pituitary malfunction cause low level production of LH, FSH (Maclean and Lee, 1999; Jacobs,2002; Marrison *et al*., 2004), oestrogen, progesterone (Jacobs, 2002), but, prolactin elevation (Maclean and Lee, 1999; Broadbear *et al*., 2004). Excessive prolactin secretion that is unrelated to childbirth switches off the hypothalamic-pituitary axis(HPA) i.e.decreases LH/FSH secretion (Jacobs,2002). These findings are in accordance with the present results.

Basic physiological differences between men and women influence their reactions to drugs. SSRIs appear to be maintained at higher blood concentrations in women than in men (Meibohm et al., 2002). This explains the incidence of effects in female rats more than in males after 10 days of paroxetine treatment.

During pregnancy, maternal serum estradiol and progesterone levels increase considerably and high levels are sustained throughout pregnancy (Rhoads and Tanner,

2003). The decrease in both hormones in the present study results from the effect of paroxetine on HPA as cited before.

Highly significant differences were observed in sperm analysis of paroxetine-treated rats compared to the control (Table 3).There was also other abnormalities as loss of head and long tail . Significant decreases in sperm count, motility, viability and function with an increase in sperm abnormalities were observed in paroxetine-treated male rats for 10, 20 and 30 days (Fig.1). This treatment revealed that sexual dysfunction complicated paroxetine therapy (Thase *et al*., 2005). Also, the reduction of LH, FSH and testosterone in the current results lead to the reduction in producing sperms (Jacobs, 2002). FSH regulates the development of Sertoli cells (involved in sperm maturation) in the testes, and the Graafian Follicles in the ovaries (Maclean and Lee,1999).

Table (4) shows significant reduction in mating indices in male rats treated with paroxetine for 10, 20 and 30 days. Also significant decrease in fertility index was observed. No male was able to induce pregnancy in untreated females after 20 and 30 days dosing. Significant decrease in pregnancy rate was observed in untreated females exposed to males treated with paroxetine for 10 days. There was only one pregnant case after mating between 10 days paroxetine -treated male and 10 days paroxetine-treated female (treatment was carried out before mating). This pregnant female died during treatment with paroxetine. The cause of death was not known.

Ashton *et al*. (1997) reported that the antidepressants caused sexual side effects in all 3 phases of the normal sexual response cycle, including decreased libido, erectile dysfunction (in men), and delayed time to orgasm or anorgasmia in men and women. In open case series of patients treated with SSRIs, orgasmic dysfunction is the most common sexual dysfunction, followed by decreased libido; arousal difficulties represent the least common form (Nofzinger *et al*.,1993; Gitlin,1994&1997).

Statistically significant increase in the number of resorbed embryos was observed in treated animals (Table 5). Morphological examination indicated that oral administration of paroxetine (0.36 mg/100g body weight) to pregnant females induced significant increase in the number of resorbed fetuses and decrease the fetal body weight and crown rump length of rat embryos (Figure 2A). These results are in accordance with the reduction in estradiol and progesterone observed in pregnant females in the present results.

Table (6) and Figure (2 B, C, D and E) show the skeletal system of the 19 days of gestation fetuses to mothers treated with paroxtine. There was statistically significant effects in the form of incomplete ossification of skull, central disc, and toes. Altshuler *et al*. (1997) reported three primary effects associated with psychotropic medications during pregnancy: teratogenicity, perinatal syndrome (neonatal toxicity) and postnatal behavioral sequelae. Teratogenicity is usually associated with structural abnormalities induced by exogenous compounds during organogenesis; thalidomide, which caused severe limb defects and other organ dysgenesis, and isotretinoin, which caused a wide variety of CNS, craniofacial, and cardiovascular defects are two examples of classic teratogens (Costa *et al*., 2004). Results from animal research suggest that maternal stress during pregnancy has adverse consequences on growth of offspring (Schneider *et al*., 1999), learning ability (Weller *et al*., 1988), and on postnatal development (Fride and Weinstock, 1984).

There is sufficient evidence that all psychotropic drugs readily cross the placenta to reach the fetus and may also be excreted in breast milk (Chisholm and Kuller, 1997; Arnon *et al*., 2000; Bar-Oz *et al*., 2000). Drugs in the fetus may have a higher unbound free fraction, easily penetrate into the brain, and undergo only limited hepatic and/or extrahepatic metabolism (Arnon *et al*., 2000; Hines and McCarver, 2002; McCarver and Hines, 2002), therefore, they are more hazardous to the fetus than the adults.

Cytogentic data obtained from treated male rats with (0.36 mg/100 g b.wt./day) for 10, 20 and 30 days are presented in

Table(7). Fifty metaphase spreads were examined in every rat. There was structural and numerical chromosomal aberrations. These chromo-somal aberrations included cetromeric attenuation, centric fusion, ring shape, end to end association, break, deletion and stikness. All chromosomal aberrations showed a highly significant increase in rats treated with paroxtine for 10 days and the increase is time- dependent. In addition, a highly significant increase in numerical chromosomal aberra-tions were observed (Figure 3). This result is in agreement with Ganguly (1994) who reported that chemicals, in general produce the highest frequency of aberration in rodents after 21 hours of treatment, which is enough to induce classical damage since biotransformation may take place within few hours. This sort of cytotoxicity was possibly caused not only by the parent compound, but, by its metabolites as well .

In the present work, the maximal percent of structural chromosomal aberrations was observed at 30 days of treatment .

Considering the deletion and centric separation, the latter originated either as terminal or interstitial deletion of chromosomes as clearly indicated by the unequal length of the two chromatids of the affected chromosomes (Gautam and Kapoor, 1991). The induction of centric fusion may be due to chromosome lesions near the pericentric region and subsequent re-union of the damaged segments of two chromosomes (Larramendy *et al*., 1980) .

In the present work, centric separations were significatly detected . Several authors reported and described such figure as resulting from chromosomal break attacking the centromeric region, thus leading to separation of the two chromatids (Galloway *et al*., 1987 and Hafez, 2003). There was also significant increase in the percent of chromosomal aberrations after paroxetine treatment that indicated a genotoxic effect of this drug .

Treatment with paroxetine remarkably enhanced the hyperdiploid cells over the control level .To the author's opinion, hyperdiploidy pointed to the influence of the drug upon the process of cell division. The occurrence of hyperdiploidy may be due to the production of hyperdiploidy cells in the next division of cells containing the affected chromosome (Ford and Roberts , 1983; Gautam and Kapoor , 1991).

The histological pictures of testis of male rats that recieved oral daily doses of paroxetine for 10,20, and 30 days are shown in (Figure 4). Clear small vacuoles, and irregular shape of seminiferous tubules were evident after 10 days of treatment . On day 20 large vacuoles and some spermat-ogonia leaving the basement membrane were seen. Whereas on day 30 treated rats showed hypertrophy of germinal cells, in addition to the findings observed on days 10 and 20. The reduced FSH, LH and testosterone levels in the serum of treated animals in the current study are good indicators of gonadal damage observed (Fig.4) and are supported by the work of Maclean and Lee (1999).

In fatal cases where poisoning is suspected, a quick sensitive and reliable method for drug analysis would be very much appreciated for the clinical control as well as helping their prompt identification in medico-legal practice.

Goeringer *et al*. (2000) investigated 28 deaths involving paroxetine and desc-ribed an analytical method involving isola-tion of the drug by liquid/liquid extraction at alkaline pH into n-butyl chloride, and analysis by gas chromatography/mass spectrometry (GC/MS). Also, Andrew *et al*. (2004) screened paroxetine by gas chromatography using a nitrogen phosph-orous detector.

Results in Tables (8 and 9) and Figures (5 and 6) showed that paroxetine concentration after a dose of 0.36 mg/100 g b.wt. was higher in the kidney than in the liver at different time periods of treatment in male and female rats.

The results also showed that parox-etine concentration in whole embryo tissue (mean $\pm$ S.E.) was 1.09±0.49 µg/100 g tissue.

Paroxetine hydrochloride is comp-letely absorbed after oral dosing and unde-rgoes first-pass metabolism in the liver before being extensively distributed into tissues (Goeringer *et al*., 2000). It is also highly bound to plasma proteins and its

elimination half-life is approximately 21 hours. The metabolism of paroxetine is accomplished in part by cytochrome P450 type CYP2D6. Additionally, paroxetine is a potent inhibitor of this enzyme and, therefore, inhibits its own metabolism leading to nonlinear kinetics (Ward and Azzaro, 2004; GlaxoSmithKline, 2005).

The liver and kidney have a high capacity for binding a multitude of chemicals. The two organs probably concentrate more toxicants than do all the other organs combined (Rozman and Klaassen ,2001).

The kidney tissues showed higher concentration of paroxetine (Table 8) than the liver. Rozman and Klaassen (2001) reported that the kidneys receive about 25% of the cardiac output, about 20% of which is filtered by the glomeruli. The degree of plasma protein binding affects the rate of filtration because protein- xenobiotic complexes are too large to pass through the pores of the glomeruli. About 64% of the dose is excreted in urine with 2% as the parent compound and 62% as metabolites (inactive) over 10- days post-dosing period (GlaxoSmithKline, 2005).

Meibohm *et al*.(2002) reported that selective serotonine reuptake inhibitors (SSRI) appear to be maintained at higher blood concentration in women than in men. It is thought that the concentration differences are related to gender-related CYP liver enzymes. This explains the variation in paroxetine disposition in kidney and liver of male and female rats (Tables 8&9).

Paroxetine was detected in rat embryo but in small concentration, this result agreed with Hostetter et al. (2000) who detected the antidepressant and its metabolites in all amniotic fluid samples, though the concentration of the parent compound in maternal serum was higher than that in the umbilical cord blood. They concluded that the presence of these antidepressants in amniotic fluid suggests that fetal exposure to these medications is continual and may occur through a variety of paths, thus accounting for increased fetal exposure. These paths include circulatory via placental passage, gastrointestinal via fetal swallowing, and respiratory secondary to fetal lung absorption.

Also, Duijvestijn *et al*. (2003) mentioned that the capacity of paroxetine to cross the placenta is illustrated by reports of neonatal symptoms associated with maternal use of paroxetine. Withdrawal symptoms in neonates after in utero exposure to paroxetine may be relatively slow compared with adults, due to possible immaturity of the liver in the newborn child (Nijhuis *et al*., 2001 and Nordeng *et al.*, 2001)

The present data suggest that paroxetine medication induced changes in the reactivity of hypothalamic-pituitary-gonadal axis leading to alteration in endocrine secretion which affect the reproductive function in male and female rats. The data also revealed the increased risk of congenital malformation after prenatal exposure to paroxetine.

Clinically, therefore, paroxetine is advised to be avoided during conceive trails and in pregnancy. Before prescribing paroxetine thoughtful weighing of its risk on sexual response in adults and on prenatal exposure of fetuses versus the risk of disease relapse following its discontinuation should be considered.

Table(1): Effect of paroxetine on serum concentration of pituitary gonadotrophins and sex hormones in male rats.

Parameters / Groups	Luteinizing Hormone ml U/ml	Follicle Stimulating Hormone ml U/ml	Prolactin ug/ml	Testosterone ug/ml	Progesterone ng/ml	Estradiol Pg/ml
Control	2.4± 0.03	2.2± 0.01	3.0± 0.04	3.5± 0.09	0.25± 0.01	41± 0.98
10 days	2.35± 0.02	2.3± 0.03	3.3± 0.06	3.1± 0.10	0.24± 0.01	40± 1.10
20 days	1.3± 0.04**	1.4± 0.02**	4.5± 0.05**	2.2± 0.10**	0.26± 0.02	48± 1.00**
30 days	0.99± 0.01**	1.0± 0.01**	4.9± 0.06**	1.8± 0.09**	0.26± 0.01	50± 1.20**

*= Significant compared to control ($p<0.05$)
**= Significant compared to control ($p<0.01$)

Table(2): Effect of paroxetine on serum concentration of pituitary gonadotrophins and sex hormones in female rats.

Parameters / Groups	Luteinizing Hormone ml U/ml	Follicle Stimulating Hormone ml U/ml	Prolactin Ug/ml	Testosterone ug/ml	Progesterone ng/ml	Estradiol pg/ml
Control	7.9± 0.13	7.3± 0.11	3.8± 0.09	0.2± 0.01	22± 0.8	90± 2.3
10days Before mating	6.0± 0.11*	6.1± 0.09*	4.9± 0.10*	0.89± 0.02**	16± 0.3 *	70± 1.1**
Control Pregnancy	3.1± 0.02	3.2± 0.02	270± 3.6	0.2± 0.01	150± 2.0	19000± 19.3
5-19 days pregnancy	2.9± 0.01	2.85±0.01	265± 3.0	0.21± 0.02	105± 1.2-*	15000±20.5**

*= Significant compared to control ($p<0.05$)
**= Significant compared to control ($p<0.01$)

Table(3): Effect of paroxetine on sperm count and sperm abnormalities in male rats.

Treatm-ents	Number of Examined rat	Number of Examined sperm/rat	Sperm with Abnormal head		Viability		Normal sperm		Sperm count X10^6
			Mean± S.E.	%	Mean± S.E.	%	Mean± S.E.	%	Mean± S.E. P
Control male	5	1000	105.25 ± 1.23	10.5	645.3 ± 5.6	64.5	894.5 ± 1.23	84.4	55.8±1.4
10 days	5	1000	203 ± 1.61	20.3	95.2 ± 0.4	9.5	797 ± 1.61	79.7	36.6±1.2**
20 days	5	1000	289 ± 1.20	28.9	78.1 ± 0.7	7.8	711± 1.20	71.1	27.1±1.6 **
30 days	5	1000	320 ± 2.10	32.0	69.4 ± 0.3	6.9	680 ± 2.10	68.0	17.5 ± 1.5**

** = Significant compared to control ($p<0.01$)

Table(4): Effect of paroxetine on reproductive performance %.

Animals	Mating index		Fertility index	
Female / Male treatment	Control	10 days treatment of females before mating	Control	10 days treatment of females before mating
Control	(10/10) 100%	(6/10) 60%	(10/10) 100%	(6/10) 60%
10 days	(4/10) 40%	(2/10)20%	(2/10) 20%	(1/10) 10%died
20 days	0/10 (0.0%)	0/10 (0.0%)	0/10 (0.0%)	0/10 (0.0%)
30 days	0/10 (0.0%)	0/10 (0.0%)	0/10 (0.0%)	0/10 (0.0%)

Table (5) Effect of paroxetine on fetuses.

Fetuses / Mating	Alive Mean± S.E. P	Resorbed Mean± S.E. P	Body Weight (g) Mean± S.E. P	Crown – rump Length (Cm.) Mean± S.E. P
Control Male X Control Female	8.4± 0.3	0.0± 0.0	4.6 ± 0.07	3.8 ± 0.02
Control Male X Treated Female before mating; after pregnancy	5.8 ± 0.6 **	0.5 ± 0.01 **	2.6 ± 0.06 **	2.3 ± 0.04 **
Control Male X Treated Female after Pregnancy	6.2 ± 0.5 *	0.8 ± 0.02 **	2.5 ± 0.03 **	2.3 ± 0.05 **
Treated male 10 days X control Female	7.1 ± 0.2	0.3 ± 0.01 **	3.1 ± 0.05 **	2.4 ± 0.03 **
Treated male 10 days X Treated Female after pregnancy	4.5 ± 0.4 **	1.1 ± 0.04 **	2.1 ± 0.06 **	1.9 ± 0.02 **

* = Significant compared to control ($p<0.05$)
** = Significant compared to control ($p<0.01$)

Table(6) : Teratogenic effect of paroxetine on the skeletal system of fetuses at 19 days of gestation.

skeletal defect	% of examined fetuses			
	Control MaleX Treated Female after pregnancy	**Control Male X Treated Female Before mating and after Pregnancy**	**Treated male 10 days X Control Female**	**Treated male 10 days X Treated Female After pregnancy**
Skull				
Incomplete ossification	45.11	53.22	22.14	60.21
Complete ossifcation	45.89	46.78	77.86	39.79
Sternebrae				
Fused	9.31	13.12	10.20	43.25
Absence	11.72	15.86	7.10	19.45
Normal	78.97	71.02	82.7	37.30
Ribs				
Incomplete ossification	22.58	39.53	12.91	40.72
Complete ossifcation	77.42	60.47	87.09	59.29
Vertebrae Centra				
Absence	33.24	58.71	18.40	74.38
Scoliosis	14.03	18.06	22.01	9.21
Normal	52.73	23.23	59.59	16.41
Fore Limbs				
Incomplete ossification	67.27	74.82	22.97	76.23
Complete ossifcation	32.73	25.18	77.03	23.77
Hind Limbs				
Incomplete ossifcation	71.82	75.73	23.80	71.68
Complete ossifcation	28.18	21.47	67.20	28.32

Results are expressed of means (± standard error) of ten fetuses.

Table (7) Chromosomal aberration of the bone marrow cells of male rats teated with paroxetine (0.36mg/100g b.wt.)

Chromosomal aberrations	**Control Mean± S.E.**	**Days of treatement**		
		10 days Mean± S.E. P	**20 days Mean± S.E. P**	**30 days Mean± S.E. P**
a- Structural aberration				
Break	0.56 ± 0.12	1.5 ± 0.11 **	2.8 ± 0.20 **	3.9 ± 0.21 **
Stickiness	0.85 ± 0.04	2.8 ± 0.16 **	3.1 ± 0.11 **	6.3 ± 0.16 **
Centromeric attenuation	1.01 ± 0.06	2.2 ± 0.12 **	2.9 ± 0.21 **	5.8 ± 0.14 **
Centric fusion	0.41 ± 0.05	1.4 ± 0.15 **	3.0 ± 0.18 **	6.1 ± 0.11 **
End to End	0.31 ± 0.01	2.1 ± 0.08 **	3.5 ± 0.16 **	7.4 ± 0.13 **
Deletion	0.20 ± 0.03	1.1 ± 0.09 **	2.2 ± 0.13 **	4.2 ± 0.01 **
Ring - Shaped	0.18 ± 0.01	2.0 ± 0.01 **	3.0 ± 0.12 **	6.1 ± 0.06 **
Total aberration	3.15 ± 0.32	12.1 ± 0.72 **	20.5 ± 1.11 **	47.2 ± 0.82 * *
b- Numerical aberration				
Hyperploidy	0.0	1.3 ± 0.12 **	2.3 ± 0.15 **	6.1 ± 0.31 **

Results are expressed of means (± standard error) of ten rats ;

*= Significant from control ($p<0.05$)

**= Significant from control ($p<0.01$)

Table (8) Mean concentration of paroxetine (µg/100g tissue) in liver and kidney tissues of male rats

Male Organ	10 days	20 days	30 days
Liver	18.66±2.90	24.12±2.67	32.46±4.00
Kidney	62.08±5.81	64.22±7.45	73.26±6.98

Table (9) Mean concentration of paroxetine (µg/100g tissue) in liver and kidney tissues of female rats

Female organ	10 days before mating	19 days of gestation
Liver	21.53±3.9	28.67±2.60
Kidney	59.61±4.2	61.93±4.30

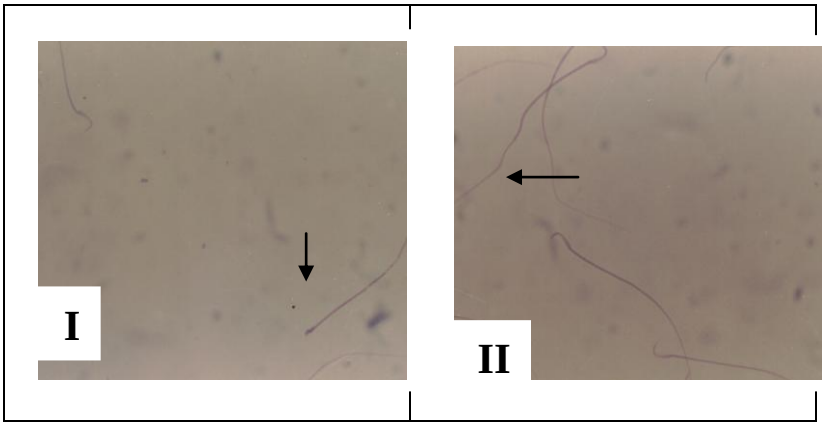


Fig (1) photographs of rat sperm smear showing abnormalitis in head and in tail I Abnormalitis in head II Abnormalitis in tail (X 250)

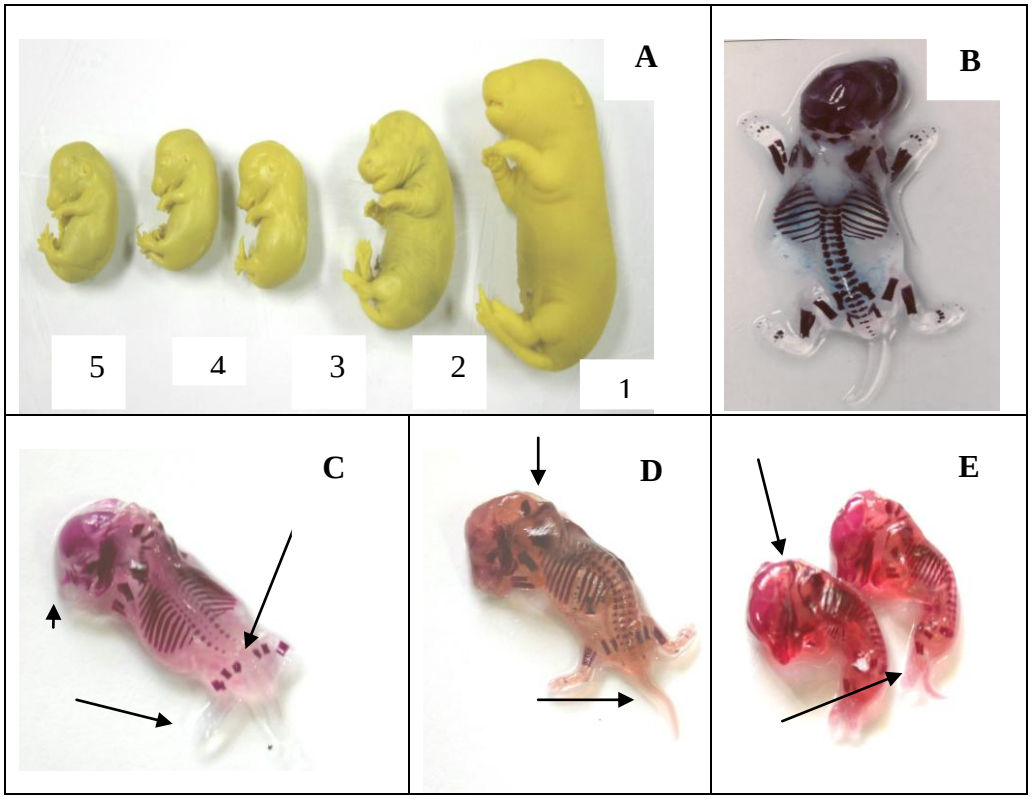


Fig (2) Photographs of control fetus and fetuses maternally treated with paroxetine showing retardation of growth and incomplete ossefecation of bones A I – Control rat fetus A 2 – Rat fetus from treated male and control female A 3 - Rat fetus from control male and treated female 10 days before mating and during gestation A4 - Rat fetus from control male and treated female during gestation. A5 - Rat fetus from treated male and treated female durin gestation. B- Control skeletal system of 19 days rat fetus C- Skeletal system of rat fetus from control male and treated female during gestation. D- Skeletal system of rat fetus from treated male and control female E – Skeletal system of rat fetus from treated male and treated female during gestation and rat fetus from treated male and treated female for 10 days before mating and during gestation. (X 1:1)

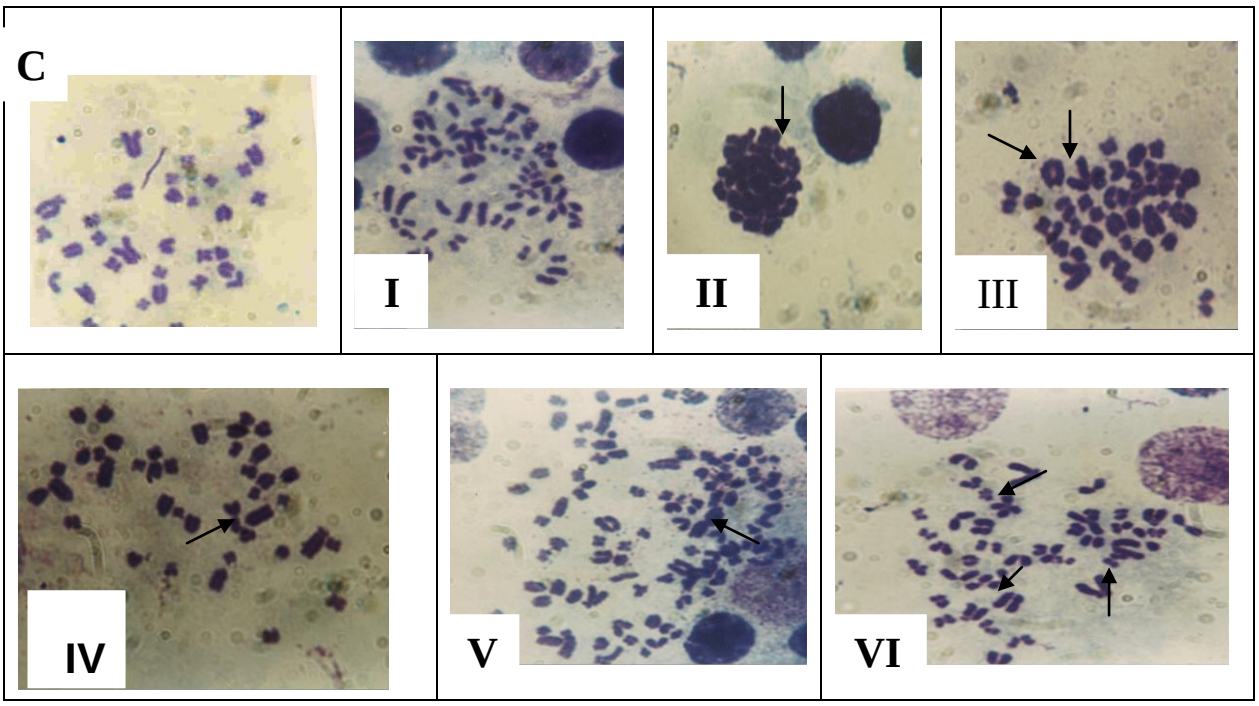


Fig (3) Photographs of bone marrow cells metaphase spread of normal and paroxetine treated male rats with 0.36 mg/100g b.w. showing C : Normal metaphase spread. I : Centromeric attenuation. II : Stickness. III : Ring shape and deletation. IV: End to end. V: Centric fusion. VI: Break and centric fusion. (X 1250)

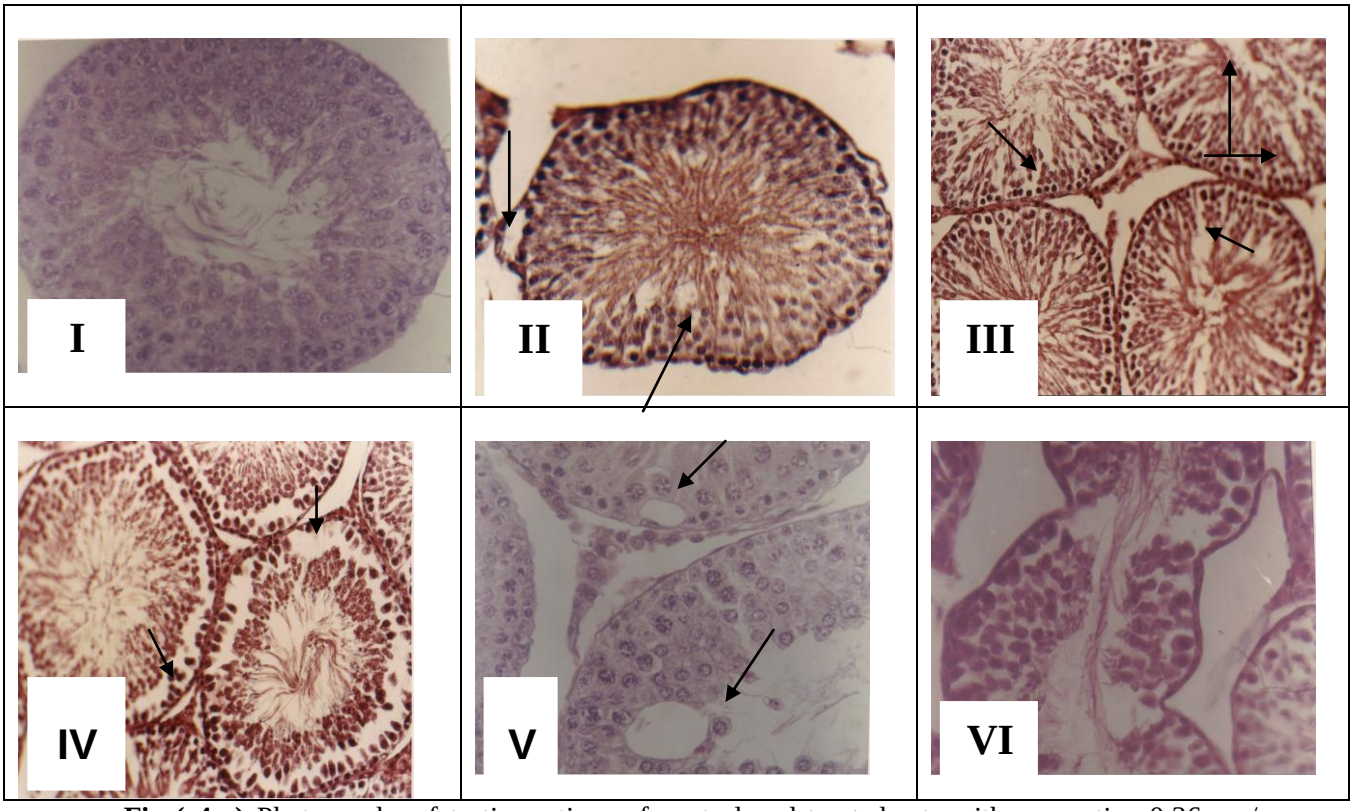


Fig (4) Photographs of testis sections of control and treated rats with paroxetine 0.36 mg/ 100g b.w. showing I: Control testis II: Spermatogonia leaving the basement membrane III: Vacoules and germinal celles degenration IV : Distortion of germinal cells V: Large vacoules and spermatogenic arrest. VI: Irregular shape of seminiferous tubules (X 250)

Fig (5) Shows paroxetine concentration in liver and kidney tissues of male rats

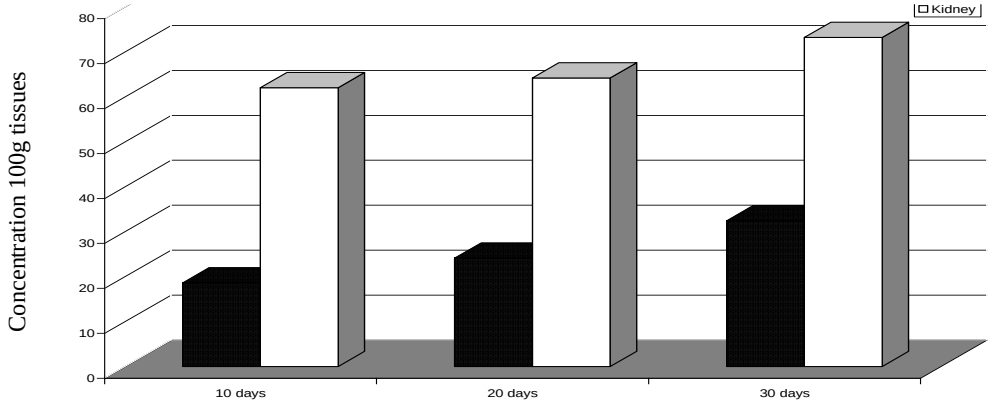
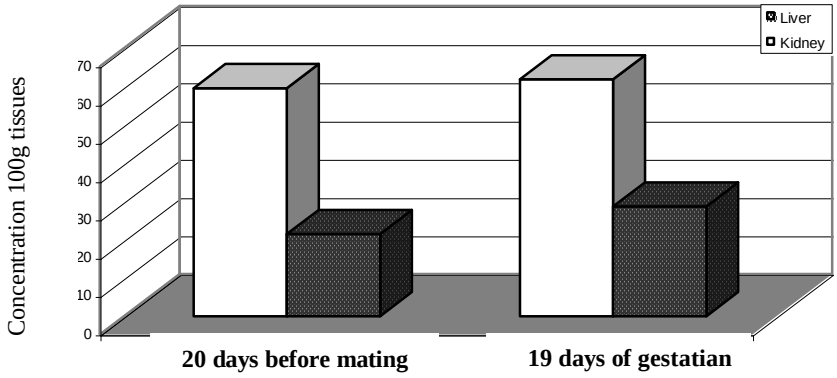


Fig (6) Shows paroxetine concentration in liver and kidney tissue female rats



References:

1. **Altshuler,L.L.;L.Cohen; M.P.Szuba ;V.K. Burt ; M. Gitlin and J.Mintz (1996):** Pharmacological management of psych-iatric illness during pregnancy: dilemmas and guidelines. Am. J. Psychiatry, 153: 592-606.
2. **Altshuler, L.L.; L.Cohen ; M.P.Szuba ;V.K. Burt ; M. Gitlin and J.Mintz (1997):** Pharmacological management of psych-iatric illness during pregnancy: dilemmas and guidelines (comment). Am. J. Psychiatry, 154(5): 718-719.
3. **Andrew C. L. ; Peter M. Marzuk; Kenneth Tardiff, and Jedediah J. Teres. (2004):** Paroxetine, other antidepressants, and youth suicide in New York city: 1993 through 1998.J .Clin. Psychiatry, 65:7.
4. **Arnon, J.; S. Schechtman, and A. Ornoy (2000):** The use of psychiatric drugs in pregnancy and lactation. Isr. J. Psychiatry Relat. Sci., 37: 205-222.
5. **Ashton, A.K.; R.Hamer, and R.Rosen (1997):** Serotonin reuptake inhibitor-induced sexual dysfunction and its treatment: A large-scale retrospective study of 596 psychiatric outpatients. J. Sex Marital Ther., 23(3):165-175.
6. **Bar-Oz, B.; I. Nulman, G.Koren, and S.Ito (2000):** Anticonvulsants and breast feeding: a critical review. Paediatr. Drugs ,2: 113-126.
7. **Broadbear, J.H.; L.C. Hutton; I.J. Clarke, and B.J. Canny(2004):** Sex differences in the pituitary- adrenal response following acute antidepressant treatment in sheep. Psychopharmacology (Berl),171(4): 450-457.
8. **Chisholm, C.A., and J. A.Kuller (1997)** :A guide to the safety of CNS-active agents during breastfeeding.Drug Saf.,17:127-142.
9. **Costa, L.G.; L. Steardo, and V. Cuomo (2004) :** Structural effects and neurofun-

ctional sequelae of developmental exposure to psychotherapeutic drugs: experimental and clinical aspects. Pharmacol. Rev., 56:103-147.

10. **Daoud,A.S.; H.Bataineh; S.Otoom, and E. Abdul-Zahra (2004):** The effect of vigabatrin, lamotrigine and gabapentin on the fertility, weight, sex hormones and biochemical profiles of male rats, Neuro-Endocrinol. Lett.,25(3):178-183.
11. **Drury, R.A., and E.A. Wallington (1980):** Carletons histological technique. 5th ed. Oxford University, London, New York, Toronto, pp. 140-142.
12. **Duijvestijn, C. M.; Kalmeijer, D.; Passier, L. M.; Dahlem Peter and Smiers Frans (2003):** Neonatal intraventricular haemorrhage associated with maternal use of paroxetine.Br. J. Clin. Pharmacol.,56: 581–582.
13. **Ferguson,J.M.(2001):** The effects of antidepressants on sexual functioning in depressed patients:a review. J. Clin. Psychiatry, 62(suppl 3):22-34.
14. **Ford , J.H., and Roberts , C. (1983):** Displacement of chromosomes in mitosis: a technique for assessing differential chromosomes error . Cytogenetic (Cell Genetic) , 36 : 537-541.
15. **Fride, E., and M.Weinstock (1984):** The effects of prenatal exposure to predictable or unpredictable stress on early developm-ent in the rat. Dev. Psychobiol.,17:651-660.
16. **Galloway , S.M ; Deasy , D.A. ; Bean , C.L. ; Kraynak , A.R. ; Armstrong , M.J., and Bradley , M.O.(1987):** Effects of high osmotic strength on chromosome aberrations, sister – chromatid exchanges and DNA strand breaks , and the relation to toxicity . Mut. Res., 189: 15-25 .
17. **Ganguly , B.B.(1994)** :Bone marrow clastogenicity of trimethyltin. Mut .Res ., 312 : 9-15 .
18. **Gautam , D.C. and Kapoor , I. (1991) :** genotoxic effects of dithane M – 45 on the bone marrow cells of mice in vivo . Experimenta , 47 : 280 – 282
19. **Gitlin, M.J.(1994):** Psychotropic medica-tions and their effects on sexual dysfunc-tion: Diagnosis, biology and treatment approaches.J. Clin. Psychiatry, 55:406-413.
20. **Gitlin, M.J.(1997):** Sexual side effects of psychotropic medications,Psychiatric Clin. North Am. Annual Drug Ther., 4: 61-90.
21. **Glaxo Smith kline Co.(2005):** PAXIL Research Triangle Park, NC 27709.
22. **Goeringer, K.E.; Raymon, L.; Christian G.D., and Logan, B.K.(2000):** Postmortem forensic toxicology of selective serotonin reuptake inhibitors: a review of pharmacology and report of 168 cases.J. Forensic Sci., 45(3):633-48.
23. **Grimsley, S.R. and M. W. Jann (1992):** Paroxetine, sertraline, and fluvoxamine: new selective serotonin reuptake inhibitors. Clin. Pharm., 11:930-57.
24. **Hafez,D.A. (2003):**Genotoxicity of tobramycin on somatic and germ cells of albino mice .Master Science Thesis, Girls College for Arts , Science And Education , Ain Shams university , pp.53-62 .
25. **Hendrick,V.; M. Gitlin; L. Altshuler, and S. Korenman (2000):** Antidepressant medications, mood and male fertility. Psychoneuroendocrinology, 25(1): 37-51.
26. **Herzog,G.A.; W.F. Drislane; D.L. Schomer; P.B. Pennell; E.B. Bromfield; K.M. Kelly; E.L.Farina, and Ch.A. Frye (2004):** Differential effects of antiepileptic drugs on sexual function and reproductive hormones in men with epilepsy:Interim analysis of a comparison between lamot-rigine and enzyme inducing antiepileptic drugs. Epilepsia, 45(7):764-768.
27. **Hines, R.N., and D.G. Mc Carver (2002):** The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J. Pharmacol. Exp. Ther., 300: 355-360.
28. **Hoffmann, G.R. (1996):** Genetic toxicology. In : Casarett and Doulls Toxicology; The basic Science of Poisons. Edited by Klassen, C.D., McGraw Hill, New York, 5th ed.,pp.269-300.
29. **Hostetter A.; Ritchie J.C. and Stowe Z.N.(2000):** Amniotic fluid and umbilical cord blood concentrations of antidepre-ssants in three women. Biol. Psychi-atry,48(10):1032-1034.
30. **Jacobs,H.(2002):** The function and interaction between the pituitary gland and the sex hormones. The Pituitary Foundation,Conference on hormones, sex and fertility.
31. **Koren, G., A. Pastusnak and S. Ito (1998):** Drugs in pregnancy. N. Engl. J. Med., 338: 1128-1137.
32. **Kuller, J.A.; V.L.Katz; M.J. MacMahon; S.R.Wells, and R.A. Bashford (1996):** Pharmacologic treatment of psychiatric disease in pregnancy and lactation: fetal and neonatal effects. Obstet. Gynecol., 87: 789-794 .
33. **Larromendy, M.L.; F.N.Dulout; N.O.Blanchi, and O.A.Olivero (1980) :** In vivo dose – response relationship in bone

marrow cells of mice treated with adriamycin. Mut. Res. , 79 : 133 – 140.

34. **Lee, H.S.; D.H. Song, and C.H. Kim, et al (1996):** An open clinical trial of fluoxetine in the treatment of premature ejaculation. J. Clin. Psychopharmacol., 16:379-82.
35. **Lucke, C.A., and R.R.Tice (1987):** Effect of processing time on the quality of mouse bone marrow metaphase preparations. Mut. Res., 227: 59-62.
36. **Maclean, F. and A. Lee (1999):** Drug-induced sexual dysfunction and infertility, The Pharmaceutical. J., 262 (7047): 780-784.
37. **Marrison, J.L.; K.W. Riggs; C.Chien; N. Gruber; I.C. McMillen, and D.W. Rurak(2004):** Chronic maternal fluoxetine infusion in pregnant sheep: effects on the maternal and fetal hypothalamic-pituitary-adrenal axis. Pediatr. Res., 56(1):40-46.
38. **McCarver, D.G., and R.N. Hines (2002):** The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. J. Pharmacol. Exp. Ther., 300: 361-366.
39. **Meibohm, B.; I. Beierle and H. Derendorf (2002):** How important are gender differences in pharmacokinetics?. Clin. Pharacokinet., 41(5): 329-342.
40. **Meistrich, M.L.(1989):** Evaluation of reproductive toxicity by testicular sperm head counts. J. Am. Coll. Toxicol., 8: 551-567.
41. **Michael,J.G.(1998):** Treatment of antidepressant-induced sexual dysfunction. Psychiatry & Mental Health J., 3(3):189-98.
42. **Nickolls, L.C.(1956):** The scientific investigation of crime, 1st ed., London, Butterworth, pp.382-383.
43. **Nijhuis, I.J.; Kok-Van Rooij, G.W.,and Bosschaart, A.N. (2001):** Withdrawal reactions of a premature neonate after maternal use of paroxetine. Arch. Dis. Child. Fetal. Neonatal,84: F77.
44. **Nofzinger, E.A.; M.E. Thase, and C.F. Reynolds, et al.(1993):** Sexual function in depressed men: Assessment by self-report, behavioral and nocturnal penile tumescence measures before and after treatment with cognitive behavior therapy. Arch. Gen. Psychiatry, 40:24-30.
45. **Nordeng, H.; Lindemann, R.; Perminov, K.V., and Reikvam A. (2001):** Neonatal withdrawal syndrome after in vtero exposure to selective serotonin reuptake inhibitors. Acta Pediatr.,90: 288–291.
46. **Paget, G.E.,and J.M. Barnes (1964):** Evaluation of drug activities and pharmacometrics. Editors Laurence, D.R. and Bacharach, A.L. Academic Press, London, 1:135-166.
47. **Perry, P. (2004):** Selective serotonin reuptake inhibitors and the treatment of affective illness. Clinical Psychopharmacology Seminar.
48. **Rhoades, R.A., and G.A. Tanner(2003):** Medical physiology. Lippincot Williams and Wilkins, 2nd ed., pp.664-665.
49. **Rozman,K.K. and C.D. Klaassen(2001):** Absorption, distribution and exceretion of toxicants in : Casarett and Doulles Toxicology, edited by Klaassen, C.D., McGraw Hill Medical Publishing Division, 6th ed., pp.119-125.
50. **Schneider, M.L.;E.C. Roughton, A.J.Koehler and G.R.Lubach (1999):** Growth and development following prenatal stress exposure in primates: an examination of ontogenetic vulnerability. Child Dev., 70: 263-274.
51. **Smith,S.H.(2004):** Sexual disorders and psychosexual therapy. Psychiatry, 3(2):20-23.
52. **Swaby,H.E.(1995):** A review of pregnancy outcome following exposure to newer antidepressants. University of Maryland, Drug Information Center.
53. **Thase, M.E.; B.R.Haight; N.Richard; C.B.Rockett; M.Mitton; J.G. Modell; S.Vanmeter; A.E.Harriett, and Y. Wang (2005):** Remission rates following antidepressant therapy with bupropion or selective serotonin reuptake inhibitors: a meta-analysis of original data from 7 randomized controlled trials. J. Clin. Psychiatry, 66(8): 974-981.
54. **Venitt, S., and J.M. Parry (1984):** Mutagenicity testing. A practical approach.IRL. Press, Oxford and Washington, pp.217-221.
55. **Weesner, F.M.(1986):** General zoological microtechniques. Scientific Book, Indian ed. Agency. Calcutta.pp.100-101.
56. **Ward, H.E., and A.J. Azzaro (2004):** Drugs used in mood disorders in : Modern Pharmacology with Clinical Applications edited by : Craig, C.R. and Stitzel, R.E., Lippincott Williams and Wilkins 6th ed., p.388.
57. **Weller, A.; H. Glaubman ; S.Yehuda; T.Caspy, and Y.Ben-Uria (1988):** Acute and repeated gestational stress affect offspring learning and activity in rats. Physiol. Behav., 43: 139-143.

التأثير السمى للباروكستين على وظائف الخصوبة والتكاثر فى الجرذان

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يعرض البحث لتأثير عقار الباروكستين المستخدم فى علاج بعض حالات الاكتئاب على الخصوبة وعلى الأمهات الحوامل وأجنتها. وقد استخدمت عدة معايير فى الدراسة من بينها قياسات لهرمونات الغدة النخامية PRL, FSH, LH وأيضا هرمونات الغدد التناسلية التستوستيرون والبروجستيرون والاسترادايول. هذا بالإضافة لدراسة هستوباثولوجية للخصية فى الحيوانات المعاملة بالعقار.

ولقد أوضحت النتائج أن الجرعة المستخدمة من العقار وهى 36و0مجم/100جم من وزن الجسم والتى تعادل الجرعة العلاجية فى الإنسان قد تسببت فى نقص ذو دلالة معنوية فى تركيز هرمونات FSH, LH، والتستوستيرون فى السيرم. بينما زاد تركيز البرولاكتين، وذلك فى ذكور الجرذان المعاملة لمدد 10،20،30 يوم وأن التأثير يزداد بزيادة المدة. ولقد أثرت المعالجة أيضا على الحيوانات المنوية حيث ظهر بها تشوهات بالإضافة لنقص فى عددها وحركتها. وأمتد التأثير ليشمل الأمهات المعاملة وأجنتها حيث زاد عدد الأجنة الممتصة مع نقص فى وزن وطول الأجنة بالإضافة لنقص التعظم فى هياكل هذه الأجنة. وفيما يتعلق بالكروموسومات فقد أوضحت النتائج وجود تشوهات تركيبية وعددية فى الكروموسومات.

وخلصت الدراسة الى أن الآثار الجانبية لهذا العقار ترتبط فى معظمها بالمعايير الخاصة بالغدد الجنسية وعلى وجه الخصوص الخصية وما يعكسه ذلك من تأثيرات مثبطة على السلوك الجنسى للحيوان تركيبيا على مستوى الخصية والحيوانات المنوية ووظيفيا على مستوى الخصوبة.