The Egyptian Journal of Hospital Medicine (October 2013) Vol. 53, Page 837– 845

Adverse Effects of Digoxin, as Xenoestrogen, on Some Hormonal and Biochemical Patterns of Male Albino Rats

Eman G.E.Helal *, Mohamed M.M. Badawi **,Maha G. Soliman*, Hany Nady Yousef * , Nadia A. Abdel-Kawi*, Nashwa M. G. Abozaid****

Department of Zoology, Faculty of Science, Al-Azhar University (Girls)*, Department of Biochemistry, National organization for Drug Control and Research** Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University***

Abstract

Background: Xenoestrogens are widely used environmental chemicals that have recently been under scrutiny because of their possible role as endocrine disrupters. Among them is digoxin that is commonly used in the treatment of heart failure and atrial dysrhythmias. Digoxin is a cardiac glycoside derived from the foxglove plant, *Digitalis lanata* and suspected to act as estrogen in living organisms.

Aim of the work: The purpose of the current study was to elucidate the sexual hormonal and biochemical patterns of male albino rats under the effect of digoxin treatment.

Material and Methods: Forty six male albino rats (100-120g) were divided into three groups (16 rats for each). Half of the groups were treated daily for 15 days and the other half for 30 days. Control group: Animals without any treatment. Digoxin L group: orally received digoxin at low dose equivalent of 0.0045mg/200g.b.wt. Digoxin H group: administered digoxin orally at high dose equivalent of 0.0135mg/200g.b.wt. At the end of the experimental periods, blood was collected and serum was separated for estimation the levels of prolactin (PRL), FSH, LH, total testosterone (total T), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total proteins, albumin, total lipids, total cholesterol (total-chol), Triglycerides (TG), low density lipoprotein cholesterol (LDL-chol) and high density lipoprotein cholesterol (HDL-chol).

Results: Results showed marked elevation in PRL and FSH levels and significant reduction in LH and total T levels in all treated groups compared to the corresponding controls. Serum enzyme activities (ALT, AST and ALP) and levels of urea, creatinine, total lipids, total-chol, TG and LDL-chol were obviously elevated in all the treated groups as compared to control groups. Marked decline was recorded in the values of total proteins, albumin, A/G ratio and HDL-chol in all the treated groups at the end of the two time intervals of treatment compared to controls. Regarding serum globulin level, treatment of rats with the low dose of digoxin for 15 days induced significant reduction in this parameter, while globulin returned back to its normal level after 30 days of treatment. On the other hand, the high dose of digoxin caused significant decline in serum globulin concentrations at the two time intervals of treatment. Most of the recorded changes in hormonal and biochemical parameters exhibited dose and time-dependent manner.

Conclusion: The results of the current research confirmed that digoxin disrupts the sexual hormonal pattern and biochemical parameters. So, we recommend replacing of this drug by others without estrogenic activity, particularly if it is indicated at a high dose or for a long period of time.

Key words: Xenoestrogens, digoxin, Hormones, Biochemical parameters.

Introduction:

In the last few years, increasing interest has focused on evaluating the adverse effects of endocrine-disrupting chemicals (EDCs). EDCs are a heterogeneous group of substances able to alter many endocrine functions in organisms **[1].** The mechanisms of endocrine disruptor's toxicity include direct interaction with hormone receptors as agonists or antagonists or alteration of hormone synthesis, secretion or bioavailability **[2]**. Among EDCs, xenoestrogens have been extensively studied owing to their capability to mimic natural estrogens **[3]**.

Digoxin is a cardiac glycoside derived from the foxglove plant, *Digitalis lanata* **[4].** Digitalis such as digoxin and digitoxin are commonly used in the treatment of heart

failure and atrial dysrhythmias **[5].** Digitalis has been shown to have estrogen effects on male patients.

The impact of digoxin on sexual hormonal pattern had been described in previous studies **[6-8]** but rare studies are available concerning the influence of digoxin on various biochemical indices. The purpose of the present study was to elucidate more details about the sexual hormonal and biochemical patterns of male albino rats under the effect of digoxin treatment.

Material and Methods:

Material

Digoxin was used as cardixin tablets. It was purchased from Alexandria Co. for pharmaceuticals and Chemical Industries, Egypt. Each tablet (contains 0.25 mg digoxin) was dissolved in distilled water and given orally in two therapeutic doses comparable to that given to humans on the basis of relative weight **[9].** All other chemicals used were of analytical-grade of Merck quality.

Experimental Animals

Forty six male albino rats *(Rattus norvegicus)* weighing approximately 100-120 g were obtained from *the farm of National Organization for Drug Control and research (NODCAR), Giza, Egypt.* They were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given standard diet prepared according to modified AIN-93-A **[10]** and water *ad-libitum.* Rats were maintained under standard laboratory conditions at 25±2^{o}C, relative humidity 50±15% and normal photoperiod (12h light/dark cycle).

Experimental design

After one week of acclimatization, the rats were divided randomly into three groups (16 rats for each). Half of the groups were treated for 15 days and the other half for 30 days.

- **Control group**: Animals without any treatment.
- **Digoxin L group**: Animals were received digoxin daily at low dose equivalent of 0.0045mg/200g.b.wt. through gastric tube.
- **Digoxin H group**: Animals were daily administered digoxin orally at high dose equivalent of 0.0135mg/200g.b.wt.

Blood collection

At the end of the experimental periods, rats were fasted overnight (12h) and then anesthetized through a slight diethyl ether exposure. Blood samples were collected from retro-orbital plexus and serum was obtained by blood centrifugation at 3000 rpm for 10 min at 4°C and immediately stored at -20°C till time of analysis.

Hormonal assay:

Serum PRL, FSH, LH and total T levels were measured by Enzyme-linked Immune Sorbent Assay (ELISA) according to **Liu and Zhous [11]; Urban *et al.* [12]; Levine *et al.* [13] and Bricaire *et al.* [14]** respectively.

Estimation of other biochemical parameters

The activities of serum AST and ALT were assayed by the method of **Reitman and Frankel [15].** Levels of serum ALP, urea, creatinine, total proteins and albumin were estimated according to **John [16]; Patton and Crouch [17]; Gottireid *et al.* [18]; Gornall *et al.* [19] and Corcoran and Durnan [20]** respectively. Serum total lipids **[21]**; Total-chol **[22]**; TG **[23]**; LDL-chol and HDL-chol **[24]** were estimated colorimetrically using high quality kits according to manufacturer's protocol.

Statistical analysis

The results were expressed as mean ± S.E.M of 8 rats per group and the statistical significance was evaluated by Student's *t*-test using the SPSS/17.0 software. Values were considered statistically significant when $P< 0.05$.

Results:

Effects of digoxin on sexual hormonal pattern

Table (1) shows mean concentrations of PRL, FSH, LH and total T in serum of control and treated groups. digoxin in low dose (0.0045 mg/200 g.b.wt.) and high dose (0.0135 mg/200 g.b.wt.) caused marked elevation ($P<0.01$) in PRL and FSH levels after 15 and 30 days of administration as compared to the corresponding controls. A reversed pattern was apparent for LH and total

T values where their levels were significantly decreased (P<0.01).

Effects of digoxin on other serum biochemical parameters

The data in table (2), showed that treatment of rats with digoxin induced significant increase (P<0.01) in the enzyme activities (ALT, AST and ALP) with low and high doses for both periods of treatment as compared with the control groups. The recorded elevation in all enzyme activities exhibited dose and time-dependent manner.

Serum urea and creatinine of control and digoxin-treated groups for 15 and 30 days are shown in table (3). The sera of digoxin-treated groups (both the low and high dose) had significantly (p<0.05) high levels of urea and creatinine relative to the corresponding control rats. The recorded elevation in these parameters became more obvious by increasing both the concentration of digoxin and the treatment period.

Table (4) showed that rats received digoxin exhibited significant reduction (P<0.01) of total proteins, albumin and A/G ratio levels in all the treated groups at the end of the two time intervals of treatment compared to the controls. Regarding serum globulin level, treatment of rats with the low dose of digoxin for 15 days induced significant reduction (P<0.05) in this parameter but it returned toward control levels after 30 days of treatment. On the other hand, the high dose of digoxin caused significant decline (P<0.01) in serum globulin concentrations at the two time intervals of treatment.

Table (5) showed that rats treated with digoxin exhibited a significant elevation (P<0.01) in total lipids, total-chol, TG and LDL-chol at the two time intervals of treatment compared to control rats. On contrast, HDL-chol was significantly reduced (P<0.01) in rats treated with low and high doses of digoxin at the two time intervals of treatment. The recorded elevation or decline in all the measured parameters of serum lipid profile exhibited dose and time-dependent manner

Table 1.

Effects of low and high doses of digoxin on certain serum hormonal levels of male albino rats.

	Hormones	Control group	Digoxin L		Digoxin H	
		Mean±SE	Mean±SE	% change	Mean±SE	% change
15 days	PRL (ng/ml)	4.09±0.244	5.51±0.254*	35%	7.3±0.101**	78%
	FSH (mIU/ml)	1.99±0.07	3.31±0.174**	66%	4.58±0.188**	130%
	LH (mIU/ml)	10.9±0.343	9.17±0.169**	-16%	7.88±0.34**	-28%
	Total T (ng/ml)	15.5±0.188	14.1±0.095**	-9%	12.7±0.29**	-18%
30 days	PRL (ng/ml)	4.6±0.197	6.9±0.24**	5%	9.1±0.205**	98%
	FSH (mIU/ml)	2.02±0.08	4.8±0.278**	138%	6.12±0.161**	202%
	LH (mIU/ml)	11.07±0.28	7.5±0.193**	-32%	6.42±0.186**	-42%
	Total T (ng/ml)	15.2±0.54	12.2±0.43**	-20%	10.54±0.34**	-31%

Values represent mean ± S.E. (n=8 rats).

* P<0.05, ** P<0.01 compared to the corresponding control group.

Table 2.
Effects of low and high doses of digoxin on certain serum enzymes (ALT, AST and ALP) levels (U/L) in male albino rats.

	Enzymes	Control group	Digoxin L		Digoxin H	
		Mean±SE	Mean±SE	% change	Mean±SE	% change
15 days	AST	15.3±0.80	36.7±1.28**	139%	63.3±1.98**	313%
	ALT	18±0.577	34.5±1.18**	91%	66.3±1.02**	268%
	ALP	403.5±4.02	500.2±2.96**	24%	684±2.8**	69%
30 days	AST	16.3±0.67	60.5±1.34**	271%	90.5±1.33**	455%
	ALT	18.8±0.87	45.7±0.84**	143%	81.8±1.046**	335%
	ALP	401.1±2.8	706.1±4.4**	76%	907±3.8**	126%

Values represent mean ± S.E. (n=8 rats).
** P<0.01 compared to the corresponding control group.

Table 3.
Effects of low and high doses of digoxin on biochemical parameters of renal function (urea and creatinine) "expressed as mg/dl" of male albino rats.

	Parameters	Control group	Digoxin L		Digoxin H	
		Mean±SE	Mean±SE	% change	Mean±SE	% change
15 days	Urea	38.3±0.91	51.8±1.91**	35%	69.7±1.42**	82%
	Creatinine	0.44±0.0133	0.54±0.013**	24%	0.67±0.018**	54%
30 days	Urea	40.8±1.39	64.83±1.99**	59%	88.62±1.61**	117%
	Creatinine	0.43±0.0189	0.59±0.0191**	39%	0.90±0.0171**	111%

Values represent mean ± S.E. (n=8 rats).
** P<0.01 compared to the corresponding control group

Table 4.
Effects of low and high doses of digoxin on serum protein fractions (g/dl) of male albino rats.

	Protein fractions	Control group	Digoxin L		Digoxin H	
		Mean±SE	Mean±SE	% change	Mean±SE	% change
15 days	Total proteins	8.3±0.07	5.8±0.11**	-30%	5.1±0.08**	-38%
	Albumin	5.2±0.05	3.18±0.10**	-40%	2.5±0.10**	-51%
	Globulin	3.1±0.12	2.7±0.057*	-14%	2.6±0.05**	-16%
	A/G ratio	1.67±0.05	1.17±0.036**	-29%	0.96±0.05**	-42%
30 days	Total proteins	8.2±0.09	5.3±0.12**	-36%	4±0.012**	-51%
	Albumin	5.13±0.07	2.2±0.06**	-57%	1.4±0.08**	-72%
	Globulin	3.1±0.08	3.1±0.05	0%	2.6±0.05**	-16%
	A/G ratio	1.6±0.02	0.71±0.019**	-57%	0.53±0.029**	-68%

Values represent mean ± S.E. (n=8 rats).
* P<0.05, ** P<0.01 compared to the corresponding control group.

Table 5.
Effects of low and high doses of digoxin on serum lipid profile (mg/dl) of male albino rats.

	Lipid fractions	Control group	Digoxin L		Digoxin H	
		Mean±SE	Mean±SE	% change	Mean±SE	% change
15 days	Total lipids	638±4.8	808.8±3**	27%	952±3.4**	49%
	Total chol.	192.5±1.1	239±1.8**	24%	285±1.5**	48%
	TG	175.8±2.2	245±1.99**	39%	303.5±1.76**	72%
	HDL-chol.	55±1.4	47.1±0.7**	-14%	42±1.06**	-24%
	LDL-chol.	102±2.25	143±1.53**	40%	182.9±1.59**	79%
30 days	Total lipids	638±3.5	975.5±3.8**	52%	1006±5.8**	58%
	Total chol.	194±1.6	310±2.4**	59%	349±2.7**	80%
	TG	175±2.2	337±2.0**	92%	376±3.5**	114%
	HDL-chol.	55±1.44	40.5±0.76**	-26%	38.6±0.71**	-30%
	LDL-chol.	103.9±1.2	202.9±1.99**	95%	235.4±2.7**	126%

Values represent mean ± S.E. (n=8 rats).
** P<0.01 compared to the corresponding control group.

Discussion:

Digitalis cardiac glycosides, such as digoxin and digitoxin, are clinically used to increase cardiac contractility in congestive heart failure **[25].** The purpose of the current study was to investigate the sexual hormonal and biochemical patterns of male albino rats under the effect of digoxin treatment.

Results demonstrate that subjecting of rats to the two dose levels of digoxin caused marked elevation in PRL and FSH levels with concomitant reduction in LH and total T values at the two time intervals of treatment. The recorded change in the measured hormonal levels became more obvious by increasing both the concentration of digoxin and the treatment period.

Endocrine-disrupting chemicals may disrupt endocrine homeostasis via interactions with endogenous hormone pathways **[26-28].** In particular, a class of endocrine disruptors, xenoestrogens, appears to trigger cellular responses that are normally induced by sex steroids, although their mode of action is still unclear **[28-30].**

PRL is a pituitary hormone and it has a variety of effects on many physiological systems such as growth and development, endocrinology and metabolism, brain function and behavior, reproduction, and immuno-regulation **[31].** The disturbance in PRL production could affect the whole organism **[31].** Despite this potential, there are relatively few published studies that have investigated the effects of xenoestrogens on PRL production **[32-35].** The elevation in PRL production recorded in the current study is in accordance with previous results obtained by **Steinmetz *et al*. [33]; Chun and Gorski [34] and Rousseau *et al*. [35]** using other xenoestrogens. **Rousseau *et al*. [35]** suggested that xenoestrogens could indeed modulate an estrogen-inducible gene such as PRL, possibly acting via second messenger-mediated cellular mechanisms instead of solely competing with estrogens for the nuclear estrogen receptor sites. Gynecomastia in elderly men had been noted in association with digoxin therapy **[36.].** This can be attributed to the estrogenic effect of digoxin that stimulates high production of PRL as PRL expression and secretion are known to be under estrogenic control **[37&38].**

Differences in the response of FSH and LH to digoxin could be due to differential sensitivity of the systems regulating FSH and LH secretion to digoxin at the level of the pituitary or the hypothalamus **[39].** Estrogens exert their biological role by binding to estrogen receptor (ER). Both ER types, α and β, can be found widely in the male reproductive system. xenoestrogens can bind to ER and exert effects to some extent similar to natural estrogens. One of the possible explanations of the disturbance in the levels of FSH, LH and total T is that high levels of estrogen may cause a reduction in both Gonadotropin-releasing hormone (GnRH) secretion and pituitary responsiveness to GnRH **[40].** However, the direct effect of estrogen on testicular cells cannot be ruled out. Estrogen directly can retard pubertal Leydig cells development **[41]** and inhibit testosterone production **[42&43].** The current results are in agreement with the results of **Lin *et al* [8] and Wang *et al*. [44]** who suggested that digoxin inhibits the production of testosterone in rat testicular interstitial cells, at least in part, via attenuation of the activities of adenylyl cyclase and cytochrome $P450_{scc}$.

Serum AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases **[45].** During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood. Their quantification in blood is useful biomarker of the extent and type of hepatocellular damage **[46].** Data from the present study showed that digoxin caused hepatocyte injury with a significant increase in serum levels of AST and ALT. This injury exhibited dose and time-dependent manner. Serum ALP level is also related to the status and function of hepatic cells. Digoxin administration in the present study also caused significant increase in the serum ALP which may be due to increased synthesis in presence of increasing biliary pressure **[47].** Our results comply with **Wójcicki [48]** who reported that digoxin is predominantly eliminated via the kidneys, metabolized in the liver, secreted into the bile or participating in the enterohepatic circulation. The changed pharmacokinetics of such drugs, in the case of mechanical jaundice, may be due to an altered liver status which can affect the function of the kidney

Elevated creatinine and blood urea nitrogen levels are likely evidence of impaired kidney functions. The obtained results showed high levels of urea and creatinine in sera of digoxin-treated groups relative to the corresponding control rats. After administration of digoxin, 50-70% of the dose is excreted unchanged **[49].** This may indicate that, digoxin exhibit adverse effects on the kidney functions. These findings coincide with those of **Okada *et al.* [50]** but contradict with the results obtained by **Cappuccio *et al.* [51]** who recorded no changes in blood urea and creatinine in response to digoxin treatment or it may be a dose dependent.

Regarding the proteins profile, results showed significant reduction in total proteins, albumin and A/G ratio levels in all the treated groups throughout the two time intervals of treatment compared to the controls. The liver is the main site of the conjugation and detoxification of drugs and other foreign substances **[52].** The hypoproteineamia and depressed albumin synthesis observed in the present study revealed the hepatotoxic nature of digoxin on liver cells. Digoxin is a steroid-like structure, that means it can readily cross the plasma membrane and inhibits protein synthesis through binding directly to ribosomes forming an inactive complex **[53].** Moreover, plasma of patients with liver cell damage often shows a decrease in the A/G ratio **[54].**

According to the results of biochemical evaluation, digoxin caused significant elevation in total lipids, Total-chol, TG and LDL-chol with concomitant reduction of HDL-chol. Endogenous as well as exogenous estrogens can affect lipoprotein metabolism **[55].** Estrogens affect the lipid profile by actuating hepatic expression of genes involved in lipoprotein metabolism **[56&57].**

Depending on the results of the current research we can conclude that digoxin disrupts the sexual hormonal pattern and biochemical parameters. So, we recommend replacing of this drug by others without estrogenic activity, particularly if it is indicated at a high dose or for a long period of time.

References:

1. **Neubert D. (1997):** Vulnerability of the endocrine system to xenobiotic influence. Regul. Toxicol. Pharm., 26:9-29.
2. **Committee on Hormonally Active Agents in the Environment (1999):** Hormonally Active Agents in the Environment. National Research Council. National Academy Press, Washington DC.
3. **WHO/IPCS, (2002):** World Health Organization/International Petroleum Chemical Safety, Global assessment of the state-of-the-science of endocrine disruptors. In: Damstra, T., Barlow, S., Bergman, A., Kavlock, R., Van der Kraak, G. (Eds.), WHO/PCS/EDC/02.2. World Health Organization, Geneva, Switzerland, <http://ehp.niehs.nih.gov/ who/>.
4. **Hollman A. (1996):** Digoxin comes from Digitalis lanata. B.M.J., 312: 912.
5. **Smith TW. (1988):** Digitalis: mechanisms of action and clinical use. New Engl. J. Med., 318:358-365.
6. **Tappler B, Katz M. (1979):** Pituitary-gonadal dysfunction in low output cardiac failure. Clin. Endocrinol. 10:219-226.
7. **Neri A, Zukerman Z, Aygen M, Lidor Y and Kaufman H. (1987):** The effect of long-term administration of digoxin on plasma androgens and sexual dysfunction. J Sex Marital Ther. 13(1):58-63.
8. **Lin H, Wang SW, Tsai SC, *et al.* (1998):** Inhibitory effect of digoxin on testosterone secretion through mechanisms involving decreases of cyclic AMP production and cytochrome $P450_{scc}$ activity in rat testicular interstitial cells. Br J Pharmacol. 125:1635-1640.
9. **Paget GE, Barnes JM. (1964):** Evaluation of Drug activities-Pharmacometrics. In: Laurence DR, Bacharach AL, editors. Toxicity tests. London, Academic Press; p. 135.
10. **Reeves PG, Nielsen FH and Fahey GC. (1993):** Ain-93 penfield diet for laboratory rodents. Nutr. 123:1939-1951.
11. **Liu MY and Zhous TT. (1994):** Radio receptor assay for human prolactin and the heterogeneity of prolactin in the sera form patients with pituitary prolactin-secreting adenoma. Chin .J. Pathophysiol. 10:420-429.
12. **Urban RJ, Evans WS, Rogol AD, *et al.* (1988):** Contemporaey aspects of discrete peak-detection algorithms. 1. The paradigm of the luteinizing hormone plus signal in man. Endocr. Rev. 9:33-37.
13. **Levine JE, Norman RL, Gliessman PM, *et al.* (1985):** In vivo gonadotrophin-reasing hormone release and serum luteinizing hormone measurements in ovariectomized, estrogen-treated rhesus macaques. Endocrinolgy. 11:707-721.
14. **Bricaire C, Raynaud A, Benotmane A, *et al.* (1991):** selective venous catheterization in the evaluation of hyperandrenism. J. Endocrinol Invest., 14: 949-956.

15. **Reitman S and Frankel S. (1957):** Colourimetric method for the vitro determination of GOT and GPT in serum or plasma. Am. J. Clin. Path. 28: 56-63.
16. **John DB. (1982):** Clinical Laboratory Methods. C.V. Mosby Co., USA, 9th Ed. P.580-581.
17. **Patton CJ and Crouch SR. (1977):** Enzymatic determination of urea (according to the urease modified Berthelot reaction). Anal. Chem. 49:464-469.
18. **Gottireid SP, Erdman GL and Amer J. (1951):** Quantitative colorimetric determination of creatinine in serum or urine. Pathol. 21: 188.
19. **Gornall AG, Bardawill CJ and David MM. (1949):** Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751-766.
20. **Corcoran RM and Durnan SM. (1977):** Albumin determination by a modified bromcresol green method. Clin Chem. 23:765-766.
21. **Kaplan A (1984):** Quantitative Determination of total lipids. Clin. Chem. The C.V. Mosby Co.St Louis. Toronto. p. 919.
22. **Henry RJ, Cannon DC and Winkelman JW. (1997):** Clinical Chemistry Principles and Tetchiness, Harper and Row. New York, p. 1440.
23. **Fossati P and Principe L. (1982):** Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chem. 28: 2077-2080.
24. **Burstein M. (1970):** Rapid method for Isolation of lipoproteins from human serum by precipitation with poly-anion. J Lipid Res.11:583-583.
25. **Iisalo E. (1977):** Clinical pharmacokinetics of digoxin. Clin Pharmacokinet. 2:1-16.
26. **Korach KS, Davis VL, Curtis SW and Bocchinfuso WP. (1997):** Xenoestrogens and estrogen receptor action. In "Endocrine Toxicology" (Thomas and Colby, Eds.), 2nd ed., pp. 181-212. Taylor & Francis, Washington, DC.
27. **Massaad C, Coumoul X, Sabbah M, *et al.* (1998):** Properties of overlapping EREs: Synergistic activation of transcription and cooperative binding of ER. Biochemistry. 37:6023-6032.
28. **Sonnenschein C and Soto A. (1998):** An updated review of environmental estrogen and androgen mimics and antagonists. J. Steroid Biochem. Mol. Biol. 65:143-150.
29. **Nesaretnam K, Corcoran D, Dils RR and Darbre P. (1996):** 3,4,3´,4´-Tetrachlorobiphenyl acts as an estrogen in vitro and in vivo. Mol. Endocrinol. 10:923-936.
30. **Petit F, Le Goff P, Cravedi JP, Valotaire Y and Pakdel F. (1997):** Two complementary bioassays for screening the estrogenic potency of xenobiotics: Recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. J. Mol. Endocrinol. 19:321-335.
31. **Bole-Feysot C, Goffin V, Edery M, Binart N and Kelly PA. (1998):** Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phonotypes observed in PRL receptor knockout mice. Endocr. Rev. 19:225-268.
32. **Wade MG, Desaulniers D, Leingartner K and Foster WG. (1997):** Interactions between endosulfan and dieldrin on estrogen-mediated processes in vitro and in vivo. Reprod-Toxicol. 11:791-798.
33. **Steinmetz R, Brown NG, Allen DL, Bigsby RM and Ben-Jonathan N. (1997):** The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. Endocrinology. 138:1780-1786.
34. **Chun TY and Gorski J. (2000):** High concentrations of bisphenol A induce cell growth and prolactin secretion in an estrogenresponsive pituitary tumor cell line. Toxicol. Appl. Pharmacol. 162:161-165.
35. **Rousseau J, Cossette L, Grenier S and Martinoli M. (2002):** Modulation of Prolactin Expression by Xenoestrogens. Gen. Comp. Endocrinol. 126:175-182
36. **Moscovitz T, Aldrighi JM, Abrahanshon PA *et al.* (2005):** "Repercussions of digoxin, digitoxin and estradiol on the endometrial histomorphometry of oophorectomized mice". Gynecol Endocrinol. 20(4): 213-220.
37. **Rhode PR and Gorski J. (1991):** Growth and cell cycle regulation of mRNA levels in GH3 cells. Mol. Cell. Endocrinol. 82:11-22.
38. **Rhode PR and Gorski J. (1991):** Inhibitory effects of serum and stimulatory effects of estrogen on prolactin mRNA levels in GH3 rat pituitary tumor cells. Mol. Cell. Endocrinol. 82:1-9.
39. **Evans NP, North T, Dye S and Sweeney T. (2004):** Differential effects of the endocrine-disrupting compounds bisphenol-A and octylphenol on gonadotropin secretion, in prepubertal ewe lambs. Domest Anim Endocrinol. 26(1):61-73.
40. **Pinilla L, Garnelo P, Gaytan F and Aguilar E. (1992):** Hypothalamic-pituitary function in neonatally oestrogen-treated male rats. J Endocrinol. 134:279-286.
41. **Abney TO. (1999):** The potential roles of estrogens in regulating Leydig cell development and function: a review. Steroids. 64:610-617.
42. **Knobil E and Neill J. (1994):** The Physiology of Reproduction. New York: Raven Press.
43. **Kula K, Walczak-Jedrzejowska R, Slowikowska-Hilczer J and Oszukowska E. (2001):** Estradiol enhances the stimulatory effect of FSH on testicular maturation and contributes to precocious initiation of spermatogenesis. Mol Cell Endocrinol. 178:89-97.
44. **Wang SW, Lin H, Hwang JJ and Wang PS. (1999):** Inhibition of testosterone secretion by digoxin in rat testicular interstitial cells. J Cell Biochem. 74:74-80.

45. **Pari L, Kumar AN. (2002):** Hepatoprotective activity of Moringa Oleifera on antitubercular drug induced liver damage in rats. J. Med. 5:171-177.
46. **Pari L, Murugan P. (2004):** Protective role of tetrahydrocurcumin against erythromycin estolate-induced hepatotoxicity. Pharmacol. Res. 49(5):481-486.
47. **Moss DW and Butterworth PJ. (1974):** Enzymology and Medicine. Pitman Medical, London, p.139.
48. **Wójcicki M. (1996):** The effect of experimental extrahepatic cholestasis on absorption, distribution and elimination of digoxin. Ann Acad Med Stetin. 42:51-65.
49. **Hallberg P, Melhus H, Hansson LO and Larsson A. (2004):** Cystatinc vs creatinine as markers of renal function in patients on digoxin treatment. Upsala J Med Sci. 109(3): 247-253.
50. **Okada RD, Hager WD, Graves PE, Mayersohn M, Perrier DG and Marcus FI. (1978):** Relationship between plasma concentration and dose of digoxin in patients with and without renal impairment. Circulation. 58:1196-1203.
51. **Cappuccio FP, Markandu ND, Sagnella GA and MacGregor GA. (1986):** The effect of oral digoxin on sodium excretion, reninangiotensin-aldosterone system and blood pressure in normotensive subjects. Postgrad Med J. 62:265-268.
52. **Segre EJ. (1975):** Naproxen metabolism in man. J.Clin. pharmacol. 15:316.
53. **Paszkiewicz-Gadek A, Chlabicz J and Galasinski W. (1988):** The influence of selected potential oncostatics of plant origin on the protein biosynthes is in vitro. Pol J Pharmacol Pharm. 40(2):183-90.
54. **Robert K, Daryl KG, Peter A *et al.*, (2000):** Hormones of the Gonads. Harper Biochemistry, 25th edition. Appleton and Lange Publishers, USA.
55. **Knopp RH. (1988):** Cardiovascular effects of endogenous and exogenous sex hormones over a woman's lifetime. Am J Obstet Gynecol. 158:1630–1643.
56. **Knopp RH and Zhu X. (1997):** Multiple beneficial effects of estrogen on lipoprotein metabolism. J Clin Endocrinol Metab. 82:3952–3954.
57. **Mendelsohn ME and Karas RH. (1999):** The protective effects of estrogen on the cardiovascular system. N Engl J Med. 340:1801–1811.